

Prostatype[®] RT-qPCR Kit

Instructions for Use



To achieve reliable results, the instructions for use must be read carefully prior to use and followed precisely.



For professional use only



Revision 2, November 2022, Prostatype Genomics AB
(www.prostatypegenomics.com/technical-documents/)



PG0009 & PG0010



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1 Document Information

1.1 Revision History

Instructions for use version	Revision Date	Change Description
2.0	November 2022	1. Added more RNA extraction kits and qPCR instruments 2. Changed business address of Prostatype Genomics

1.2 Trademarks

The following trademarks are acknowledged:

PROSTATYPE, P-SCORE, P-SCORE WEB SERVICE, PWS are the trademarks of Prostatype Genomics AB.

All other trademarks are the property of the respective owners.

2 Name and Intended Purpose

The Prostatype RT-qPCR kit utilizes one-step reverse transcription quantitative PCR (RT-qPCR) for the assessment of the mRNA expression levels of three genes IGFBP3, F3, and VGLL3 relative to the expression level of GAPDH in total RNA extracted from formalin-fixed paraffin-embedded (FFPE) human prostate cancer tissue. The output of the Prostatype® RT-qPCR Kit may be used to calculate a risk score namely, the Prostatype score (P-score) for prognostic evaluation of newly diagnosed, localized, or metastasis-free prostate cancer (PCa) patients. The product is NOT intended for the diagnosis of PCa.

The kit is for Professional use only. For use only by trained and certified laboratory staff or medically trained and certified health care professionals. The test is to be performed manually.

3 Background

The expression levels of three genes IGFBP3 (insulin-like growth factor binding protein 3), F3 (coagulation factor III (thromboplastin, tissue factor)), and VGLL3 (vestigial-like family member 3) in RNA extracted from formalin-fixed paraffin-embedded (FFPE) core needle biopsies of human prostate cancer were assessed. This three-gene expression signature has been identified and evaluated in a Swedish cohort study of 189 prostate cancer patients that were diagnosed between 1986 and 2001. The gene signature has been shown to categorize prostate cancer patients into low-, intermediate-, and high- risk subtypes (1). A follow-up study in a new cohort of 591 patients revealed that the precision of prostate cancer survival prediction could be further improved by combining genetic data with clinical parameters including the patient's PSA value, Gleason score, and Tumor-stage at diagnosis (2).

4 Principles and Procedure

4.1 Prostatype® RT-qPCR Kit

The Prostatype® RT-qPCR Kit is used to perform a 4-plex, one-step RT-qPCR test. The kit contains all reagents required for reverse transcription, amplification, and detection with quantitative PCR.

After extraction of RNA from the FFPE tissue, the mRNA is reverse transcribed using sequence specific primer pairs for the genes IGFBP3, F3, VGLL3, and the reference gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase). Subsequently, the generated cDNA is amplified and detected using individual dual-labeled hydrolysis probes (Taqman®). The hydrolysis probes are labeled with an individual fluorophore and fluorescence quencher, which prevents fluorescence detection. During the amplification step, each probe that binds to a cDNA target is hydrolyzed due to the exonuclease function of the *Taq* polymerase and the quencher is spatially separated from the fluorophore leading to the emission of fluorescence signals at specific wavelengths.

The signals are detected by the verified qPCR instruments in specific channels. Thereby, each one of the four genes can be detected and quantified independently in a single reaction well.

As the amplicons increase exponentially with each PCR cycle, fluorescence signals increase as well and are detected in real-time. When the intensity of a particular fluorescence signal crosses a pre-defined threshold, the corresponding cycle is referred to as Crossing Point (Cp). The Cp value depends on the initial concentration of the target gene. A higher initial amount of target RNA results in a lower Cp value. Based on the Cp values of the three target genes (IGFBP3, F3, and VGLL3), and the reference gene (GAPDH), the relative target gene expression (Δ Cp) can be calculated with the following formula:

$$\Delta\text{Cp (target gene)} = \text{Cp (target gene)} - \text{Cp (GAPDH)}$$

Apart from normalization of target gene expression levels, the reference gene GAPDH functions as an internal control and its corresponding Cp value in each sample is used to assess sample validity and integrity as well as to ensure that the utilized amount of RNA in the sample was sufficient.

NOTE: When using the Prostatype® RT-qPCR Kit, it is recommended to ensure that a high percentage of cancer cells are present in the tissue used for RNA extraction. To obtain optimal, reliable results, the tissue shall contain a minimum of 50% cancer cells (3). The expression signature may mirror mechanisms underlying prostate cancer pathology strengthening the hypothesis that cancer stem cells are involved in prostate cancer initiation and progression (1, 4). The amount of tissue to be used for the Prostatype® RT-qPCR assay shall be evaluated by a pathologist or a professional with equivalent training.

NOTE: After the relative expression of the three biomarker genes has been determined and analyzed using the Prostatype® RT-qPCR Kit, the genetic data is combined with clinical parameters of the respective patient. Based on this data P-score is calculated by using the P-score Web Service (PWS). Each P-score is associated with an estimated 10-year prostate cancer-specific mortality.

NOTE: Calculating a P-score requires access to the P-score Web Service (PWS), Prostatype Genomics invites users to the PWS. Please read the separate IFU for the PWS before use (5).

5 Materials

5.1 Material Provided

Each box of **4x1 patient** Prostatype® RT-qPCR Kit contains sufficient reagents to perform expression analysis of up to four patient samples plus eight controls. Inside the kit box, there are four small independent packages. Each package can be used to perform expression analysis for one patient sample along with one positive control and one negative control, thus avoiding repeating freeze-thawing of the reagents.

NOTE: If more than 1 patient needs to be analyzed in one run the kit reagents of similar kind can be pooled after thawing. DO NOT pool reagents from different batches.

Each box of **16x1 patient** Prostatype® RT-qPCR Kit contains sufficient reagents to perform expression analysis of up to 16 patient samples plus two controls.

NOTE: If the 16-patient kit is used for a single run, 16 patient samples can be analyzed, if the kit is used for two separate runs, 14 patient samples can be analyzed; if the kit is used for three separate runs, 12 patient samples can be analyzed.

5.1.1 Contents

Table 1 Contents of the Prostatype® RT-qPCR Kit.

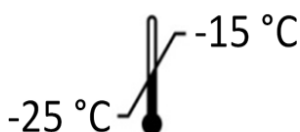
Reagent	Containers	Volume	
		4X1 kit	16X1 kit
Prostatype Master	1 vial	≥135 µL	880 µL
Prostatype Manganese	1 vial	≥ 30 µL	100 µL
Prostatype Positive Control	1 vial	≥19 µL	85 µL
Prostatype Negative Control	1 vial	≥19 µL	85 µL

5.1.2 Safety Information

When working with chemicals, always wear a laboratory coat and disposable gloves. The Prostatype® RT-qPCR Kit contains no hazardous ingredients. For more information, please consult the respective material safety data sheets (MSDS) available at our website (www.prostatypegenomics.com/technical-documents).

5.1.3 Storage and Stability

Reagents provided with the Prostatype® RT-qPCR Kit are stable until the expiration date when stored and handled as directed. Do not use material past expiration date. Do not mix components from different Prostatype® RT-qPCR Kit lots.



Store all components of the Prostatype® RT-qPCR Kit upon arrival at -25 to -15 °C.

Each component of the Prostatype® RT-qPCR Kit may be thawed and refrozen two times.

5.2 Material and Equipments to be Supplied by the User

The following laboratory materials and equipment are required to perform the Prostatype® RT-qPCR. All laboratory equipment must be installed, calibrated, operated, and maintained according to the manufacturer's recommendations.

- Disposable scalpel
- 1.5 ml or 2 ml centrifuge tubes
- Vortexer
- Pipettes with adjustable volumes of the following ranges 0.5 – 10 µL, 10 – 100 µL, 100 – 1000 µL

- Sterile, RNase-free pipet tips with aerosol barrier
- Repeater pipette or dispenser capable of repetitively dispensing volumes in an adjustable range
- 2.0 ml micro tubes with round bottom and with attached PP lid
- Microcentrifuge (with rotor for 2.0 mL tubes)
- Small shaker
- PCR Plate centrifuge
- Heating block shaker capable of incubation at 80°C
- Tips for repeat pipettes
- **For RNA extraction:** Maxwell® 16 LEV RNA FFPE Purification Kit (Promega, catalog no. AS1260), or Norgen FFPE purification kit (Norgen Biotek & Corp., Cat. 25300), or AllPrep DNA/RNA FFPE Kit (Qiagen, Cat. No. 80234), or RNeasy FFPE Kit (Qiagen, Cat. No. 73504), or PureLink™ FFPE RNA Isolation Kit (ThermoFisher Scientific, Catalog number: K156002), Roche High Pure FFPE RNA Micro Kit (Cat#04 823 125 001).
- RT- qPCR instruments: LightCycler® 480 Instrument I with 96-well heat block (Roche, catalog no. 05015278001) or LightCycler® 480 Instrument II with 96-well heat block (Roche, catalog no. 05015278001), and with Software version 1.5.x

LightCycler® 480 Multiwell Plate 96 (Roche, catalog no. 04729692001), LightCycler® 480 Sealing Foil (Roche, catalog no. 04729757001). Or BioRad CFX96 Touch (Hard-Shell® 96-Well Plates, low profile, thin wall, skirted, white/clear, cat# HSP9601), or QuantStudio™ 5 RT-qPCR System, or Applied Biosystems 7500 Fast RT -qPCR instrument (with MicroAmp™ Optical 96-well Reaction Plate with Barcode cat# 4306737 & MicroAmp™ Optical Adhesive Film, cat# 4360954).

- Disposable gloves
- DNA decontamination reagent
- RNase inhibitor

5.3 Installation Requirements

Installation, calibration, performance verification, and maintenance of the RT-qPCR Instruments need to be performed according to the manufacturer's instructions.

6 Instructions for the Preparation of Tissue Samples for Analysis with the Prostatype® RT-qPCR kit

The Prostatype kit uses RNA isolated from FFPE-prostate cancer core needle biopsies as input material. To obtain reliable results with the Prostatype® RT-qPCR test, high quality and integrity of the RNA are crucial. Adequate preparation of the biopsy material prior to RNA extraction is a critical step and includes three steps:

6.1 Sectioning of FFPE tissue blocks

From the FFPE tissue blocks, separate sections are prepared according to conventional histopathological protocols with the following modifications:

- Use DNase/RNase-free water.
- After drying the tissue section, the paraffin-melting step is omitted.

NOTE: To be suitable for use for the Prostatype® RT-qPCR Kit, FFPE tissue blocks should not exceed an age of 11 years.

NOTE: Tissue embedded in unbuffered formalin should not be used, as it does not produce reliable results.

Prepare the following sections:

- One tissue section of 5 μm thickness: This section will be used for Hematoxylin and Eosin (H&E) staining. The staining procedure shall be carried out in accordance with standard protocols.
- **Nine sequential tissue sections of 10 μm thickness:** These sections will remain unstained and the tissue will be used for RNA extraction.

NOTE: Collect the sections on glass slides without DNase/RNase contamination!

6.2 Marking and quantification of the cancer area

Using a traditional microscope or a digital scanner, mark the cancer tissue in the H&E-stained tissue section.

NOTE: Marking should be performed by a pathologist or a professional with equivalent training to avoid erroneous marking of the tumor region.

NOTE: The cancer area should be between 10 – 30 mm^2 . When marking the cancer area manually, the marked area per section should be 1.5 – 4.5 mm in length on up to 9 FFPE sections to avoid that the amount of harvest tissue is below specifications.

NOTE: To obtain good results, the marked area should contain at least 50% cancer cells.

For best results 30 mm^2 of tissue containing at least 50% cancer cell is required.

Conventional microscope:

- Mark the cancer area on the H&E-stained slide.
- Calculate the cancer area.

Digital scanner:

- Scan images at 20x magnification.
- Mark and calculate the cancer area using an image processing software.
- Transfer the cancer area marking from the scanned image to the original H&E-stained slide using a microscope.

The obtained sample serves as a map and is used as a template in the following step.

6.3 Scraping of cancer cells

In this step, the H&E-stained slide and the unstained sections are used as input material. Furthermore, a disposable scalpel and a DNase/RNase-free microcentrifuge tube are required.

- Overlap the H&E-stained section with an unstained section.
- Identify and carefully mark the cancer area on the unstained section with the scalpel blade using the H&E-stained slide as a map.
- Scrape the cancer area using the scalpel and collect the scraped tissue in a microcentrifuge tube.

NOTE: This step should be executed with high precision and a certain amount of practice is required to find the right angle when holding the scalpel in order to scrape the tissue coherently.

NOTE: A new disposable scalpel and a separate microcentrifuge tube shall be used for each sample.

NOTE: For optimal test results, we recommend scraping of 30 mm^2 tissue containing a minimum of 50% cancer cells. To achieve a sufficient amount of tissue, the pathologist can combine tumor samples from one or several biopsies of the same patient.

7 Extraction of RNA from Tissue Samples

To extract RNA from the scraped prostate cancer tissue, we recommend the use of the semi-automated

Maxwell® 16 LEV RNA FFPE Purification Kit with the instrument, or Roche High Pure FFPE RNA Micro Kit, or Norgen FFPE purification kit (Norgen Biotek & Corp., Cat. 25300), or AllPrep DNA/RNA FFPE Kit (Qiagen, Cat. No. 80234), or RNeasy FFPE Kit (Qiagen, Cat. No. 73504), or PureLink™ FFPE RNA Isolation Kit (ThermoFisher Scientific, Catalog number: K156002) according to the manufacturer's instructions with the following modifications where needed.

Suggested modifications for the Maxwell® 16 LEV RNA FFPE Purification Kit:

1. After adding mineral oil, the incubation at 80°C for 2 min is carried out under shaking at 1200 rpm.
2. Digestion of the tissue: Incubation at 56°C for 15 min (For New tissues that are used within one year, the shaking speed is 1200 rpm for 5 min, and then 650 rpm for 10 min followed by incubation at 80° for 1 hour are carried out under shaking at 650 rpm.

Suggested modifications for Roche High Pure FFPE RNA Micro Kit:

1. For specimens collected less than one year prior to RNA extraction, add 350 µL Lysis Buffer and 350 µL Binding Buffer, in the homogenization step to reduce viscosity.
2. Centrifuge the High Pure Micro filter for 4 minutes instead of 2 minutes at maximum speed before the RNA elution step.

Suggested modifications for Norgen FFPE purification kit:

1. In de-paraffinization step, shaking at 650 rpm is added during incubation at 50° C for 5 minutes.
2. In lysis preparation step, shaking at 650 rpm is added during incubation at 55oC for 15 minutes, followed by 80oC for 15 minutes.

Suggested modifications for AllPrep DNA/RNA FFPE Kit:

1. In de-paraffinization step, incubate at 56°C for 3 mins is done with shaking at 650rpm
2. In lysis step, incubate at 56°C for 15 min with shaking at 650rpm
3. Incubation of supernatant for 15 mins at 80°C with shaking at 650rpm

Suggested modifications for RNeasy FFPE Kit:

1. In de-paraffinization step, incubate at 56°C for 3 mins with shaking at 650rpm
2. In lysis step, incubate at 56°C for 15 mins with shaking at 650rpm, then at 15°C for 15 mins with shaking at 650rpm.

Suggested modifications for PureLink™ FFPE RNA Isolation Kit:

1. Shaking at 650 rpm is added in de-paraffinization step, while incubating at 72°C for 10 mins.
2. Shaking at 650 rpm is added in the lysis step during incubation at 60°C and incubation time is also prolonged to 3 hours.

NOTE: Do not store RNA after extraction and ensure that freshly extracted RNA is used in the Prostatype® RT-qPCR test within the same day.

8 Instructions for Use of the Prostatype® RT-qPCR Kit

NOTE: RNA extracted from scraped FFPE-samples is used as starting material for the Prostatype® RT-qPCR Test. Each patient sample and control shall be tested in triplicates. Δ Cp values and median Δ Cp values are calculated. Since analysis of the test is based on determining median Cp and median Δ Cp values instead of calculating mean values, outlier handling is not relevant when using the Prostatype® RT-qPCR Kit.

8.1 Setup of the RT- qPCR Instrument

Prostatype® kit was developed using Lightcycler® and this is our instrument of choice however, a list of other RT-qPCR instruments has been tested and can be used. Technical information on instrument setup is given in the following sections.

8.1.1 Saving a New Detection Format on the LightCycler® 480 Instrument I

A color compensation file needs to be saved on the LightCycler® 480 Instrument before performing the Prostatype® RT-qPCR test. This is a onetime procedure and instrument specific. General information about performing color compensation can be found in the LightCycler® 480 Instrument Operator's Manual. Contact Prostatype Genomics for information about the reagents for performing a color compensation experiment required for Prostatype® RT-qPCR Kit.

NOTE: This section describes the setup using a LightCycler® 480 Instrument I. The setup for a LightCycler® 480 Instrument II is described below.

When running the Prostatype® RT-qPCR the first time on a LightCycler® 480 Instrument I, it is necessary to set up a new detection format according to Figure 1.

- Start the software version 1.5.x.
- Click "Open Tools".
- In the tools window click "Detection Formats".
- Click "New" and give the new detection format a name, e.g. "Prostatype Channels".
- Mark the checkboxes for all of the following filter combinations:
 - Filter Combination 1: 523 – 568
 - Filter Combination 2: 558 – 610
 - Filter Combination 3: 483 – 533
 - Filter Combination 4: 615 – 670
- Change the name of the filter combinations as followed:
 - 523 – 568: GAPDH
 - 558 – 610: IGFBP3
 - 483 – 533: F3
 - 615 – 670: VGLL3
- Change the filter settings for every combination as followed:
 - Melt factor: 1
 - Quant Factor: 10

- Max Integration Time (Sec): 2

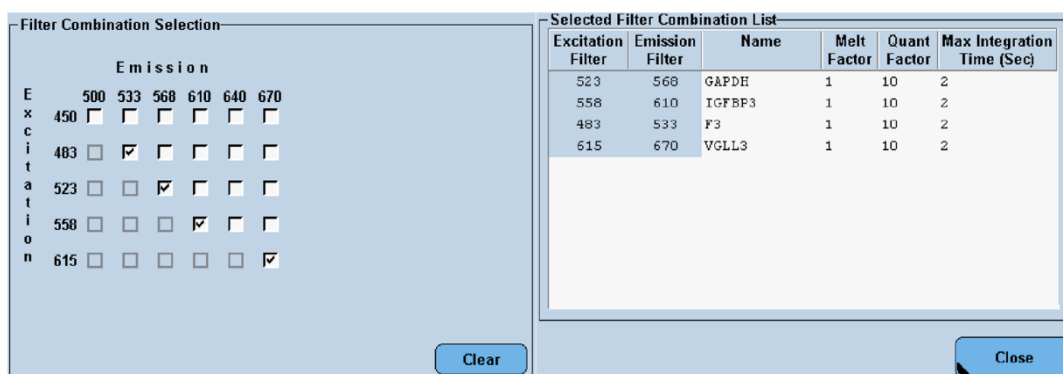


Figure 1: Filter combination for the Prostatype® RT-qPCR Kit on the LightCycler® 480 Instrument I.

8.1.2 Saving a New Detection Format on the LightCycler® 480 Instrument II

NOTE: This section describes the setup using a LightCycler® 480 Instrument II. The setup for a LightCycler® 480 Instrument I is described above.

When running the Prostatype® RT-qPCR the first time on a LightCycler® 480 Instrument II, it is necessary to set up a new detection format according to Figure 2.

- Start the software version 1.5.x.
- Click “Open Tools”.
- In the tools window click “Detection Formats”.
- Click “New” and give the new detection format a name, e.g. “Prostatype Channels”.
- Mark the checkboxes for all of the following filter combinations:
 - Filter Combination 1: 533 – 580
 - Filter Combination 2: 533 – 640
 - Filter Combination 3: 465 – 510
 - Filter Combination 4: 618 – 660
- Change the name of the filter combinations as followed:
 - 533 – 580: GAPDH
 - 533 – 640: IGFBP3
 - 465 – 510: F3
 - 618 – 660: VGLL3
- Change the filter settings for every combination as followed:
 - Melt factor: 1
 - Quant Factor: 10

- o Max Integration Time (Sec): 2

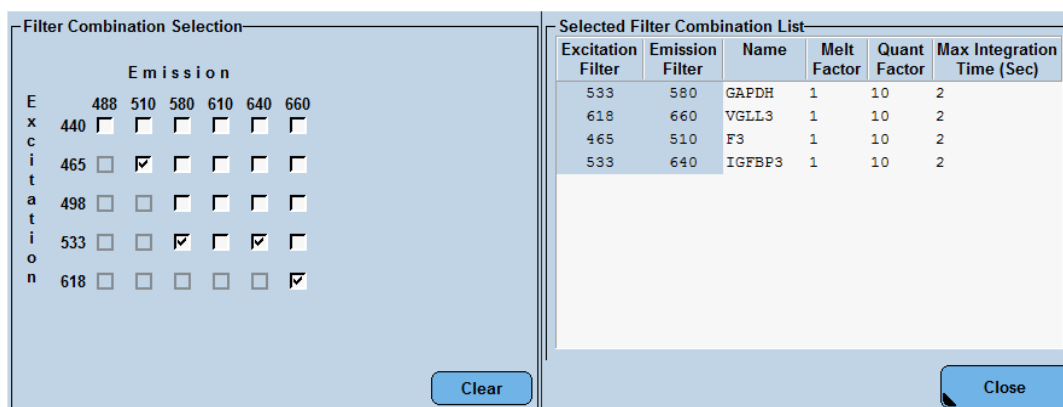


Figure 2: Filter combination for the Prostatype® RT-qPCR on the LightCycler® 480 Instrument II.

8.1.3 CFX96 Touch Real-Time PCR Detection System

This instrument does not require any color compensation as it is pre-calibrated from the manufacturer which covers the filters for Prostatype test. However, follow the manufacturers manual for color compensation during or after special situations.

Target Names can be set as bellow: GAPDH – HEX, IGFBP3 – ROX, F3 – FAM, VGLL3 - Cy5. No fifth channel/ internal reference is needed.

Table 2 RT-qPCR Program

Program Parameter	RT-Step	Cycling	
Analysis Mode	None	Quantification mode	
Cycles	1	40	
Segment	1	1	2
Target [°C]	63	92	60
Hold [hh:mm:ss]	00:20:00	00:00:05	00:00:40
Acquisition Mode	None	None	Single

8.1.4 QuantStudio™ 5 RT-qPCR System

General information about performing color compensation can be found in the instrument Operator’s Manual. Contact Prostatype Genomics for information about the reagents for performing a color compensation experiment required for Prostatype® RT-qPCR Kit.

For Prostatype run choose Experiment properties: 96-Well 0.1-mL Block >Comparative CT ($\Delta\Delta CT$) >TaqMan® Reagents > Standard run mode.

Set the experiment Method as in table 2.

Assign targets and Samples:

Quick Setup:

- Sample

- Target
- Passive Reference > None
- Reference Sample > PC (UD)
- Endogenous Control > GAPDH

Advanced Setup:

- GAPDH > VIC > Quencher NFQ-MGB
- IGFBP3 > ROX > Quencher NFQ-MGB
- F3 > FAM > Quencher NFQ-MGB
- VGLL3 > Cy5 > Quencher NFQ-MGB

8.1.5 7500 Fast Real-Time PCR System

This instrument does not require any color compensation as the pre-calibrated from the manufacturer covers the Prostatype test. However, follow the manufacturers manual for color compensation during or after special situations.

Select experiments properties: 7500 Fast (96 Wells)> Quantitation – Comparative CT ($\Delta\Delta CT$)> TaqMan® Reagents, Standard (~ 2 hours to complete a run).

Set the experiment Method as in table 2.

Define Targets:

- GAPDH > Reporter VIC > Quencher NFQ-MGB
- IGFBP3 > Reporter ROX > Quencher NFQ-MGB
- F3 > Reporter FAM > Quencher NFQ-MGB
- VGLL3 > Reporter Cy5 > Quencher NFQ-MGB

8.2 Prostatype® RT-qPCR Setup

The Prostatype® RT-qPCR Kit contains sufficient reagents to run up to 4 patient samples plus 8 controls or 16 patient samples plus 2 controls. Each RNA sample (patient RNA sample, or Prostatype Positive Control, or Prostatype Negative Control) must be tested in triplicate. Prostatype Positive Control and Prostatype Negative Control must be included in triplicate in each independent run.

NOTE: 4X1 patient kit: If more than 1 patient needs to be analyzed in one run the kit reagents of similar kind can be pooled after thawing. DO NOT pool reagents from different batches.

NOTE: Brief centrifugation of microtubes (stated as 'Briefly spin down the tubes ') is required in several steps of this instruction to remove drops from the lid or to collect remaining liquid. It is recommended to centrifuge for 10 - 20 sec at $1,000 \pm 150$ rcf using a Bench-Top centrifuge.

NOTE: Gently vortexing of tubes and containers is required in several steps of this instruction to ensure homogeneous mixing of liquid. It is recommended to use a vortexer adjusted to medium speed for 20 sec. This step is critical for proper mixing of the reagents and for obtaining valid results.

8.2.1 Thawing of Components

Thaw Prostatype Master, Prostatype Manganese, Prostatype Positive Control and Prostatype Negative Control at room temperature (15 – 25 °C) for **25 to 35 min**.

NOTE: While the components are thawing, gently vortex 4 x 20 sec to ensure proper mixing.

8.2.2 Preparation of Master Mix

NOTE: Ensure that the PCR preparation time from beginning of Mastermix preparation to starting the PCR plate does not exceed **90 min**.

NOTE: One Prostatype Positive Control and one Prostatype Negative Control must be included in each individual run.

NOTE: Each RNA sample (patient RNA sample, or Prostatype Positive Control, or Prostatype Negative Control) must be tested in triplicate.

- Gently vortex the Prostatype Master tube and the Prostatype Manganese tube. Briefly spin down.
- Transfer the corresponding volumes of Prostatype Master and Prostatype Manganese depending on the number of samples to be tested (number of patient samples + 2 controls) as indicated in Table 3 into a 2.0 mL reaction tube. (Note: For a single reaction or single well 13.61 μL of Prostatype Master and 1.39 μL of Prostatype Manganese is needed).
- Mix the Master Mix by gentle vortexing.
- Briefly spin down the Master Mix to remove drops from the lid.

NOTE: Do not store Master Mix, use immediately. All components of the Prostatype® RT-qPCR Kit may be refrozen and thawed two times.

Table 3: Preparation of Master Mix.

Number of Samples	Number of Wells in PCR Plate	Volume of Prostatype Master	Volume of Prostatype Manganese
1 sample (+2 controls)	9 wells	132.3 μL	13.5 μL
2 samples (+2 controls)	12 wells	176.4 μL	18.0 μL
3 samples (+2 controls)	15 wells	220.5 μL	22.5 μL
4 sample (+2 controls)	18 wells	264.6 μL	27.0 μL
5 sample (+2 controls)	21 wells	308.7 μL	31.5 μL
6 sample (+2 controls)	24 wells	352.8 μL	36 μL
7 sample (+2 controls)	27 wells	396.9 μL	40.5 μL
8 sample (+2 controls)	30 wells	441 μL	45 μL
9 sample (+2 controls)	33 wells	485.1 μL	49.5 μL
10 sample (+2 controls)	36 wells	529.2 μL	54 μL
11 sample (+2 controls)	39 wells	573.3 μL	58.5 μL
12 sample (+2 controls)	42 wells	617.3 μL	63.1 μL
13 sample (+2 controls)	45 wells	661.4 μL	67.6 μL
14 sample (+2 controls)	48 wells	705.5 μL	72.1 μL
15 sample (+2 controls)	51 wells	749.6 μL	76.6 μL
16 sample (+2 controls)	54 wells	793.7 μL	81.1 μL

8.2.3 Plate Preparation

NOTE: For a higher number of samples to be tested a repeater pipette/dispenser is recommended for repetitive dispensing of the Master Mix.

Set up the PCR plate as follows. A plate layout as shown in Table 4 is recommended.

- Transfer 15 μL Master Mix each into the selected wells of a LightCycler® 480 Multiwell Plate 96 Reaction Plate or other plate according to the instrument used.

- Gently vortex the patient RNA samples, the Prostatype Positive Control, and the Prostatype Negative Control. Briefly spin down.

NOTE: Positive and Negative Control vortex for 4 x 20 sec to ensure proper mixing.

- Add 5 μ L of the patient RNA samples, the Prostatype Positive Control, and the Prostatype Negative Control to respective wells of the PCR plate.
- Ensure that all samples are tested in triplicate.
- Seal the PCR plate with LightCycler® 480 Sealing Foil.
- Briefly spin down the PCR plate with a plate centrifuge for 2 min at 2000 ± 100 rcf.
- Start PCR plate within **30 min** after plate preparation.

Table 4: Recommended Plate Layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	PC	NC	NC	NC	S1	S1	S1	S2	S2	S2
B	S3	S3	S3	S4	S4	S4	S5	S5	S5	S6	S6	S6
C	S7	S7	S7	S8	S8	S8	S9	S9	S9	S10	S10	S10
D	S11	S11	S11	S12	S12	S12	S13	S13	S13	S14	S14	S14
E	S15	S15	S15	S16	S16	S16						
F												
G												
H												

PC: Prostatype Positive Control, NC: Prostatype Negative Control, S: Patient Sample

8.2.4 Plate Loading in Lightcycler® 480

NOTE: Start the PCR plate within 30 min after finalization of the plate preparation.

NOTE: It is recommended to save a run template with the defined cycling settings in the LightCycler® 480 Software.

- Start the software version 1.5.x.
- Create a new experiment, click “New Experiment”.
- Load the specified run template file or define the following experiment according to the PCR program parameters detailed in Table 5 and Figure 3 below.
- Select the detection format as set up in 8.1.1 or 8.1.2 (e.g. “Prostatype Channels”).
- Click “Subset Editor” and generate a sample subset including all used wells as described in the manual of the LightCycler® 480 Instrument.
- Enter a unique sample identifier to each of the three replicates of each sample.
- Save the experiment setup using a unique name.
- Open the tray.
- Place the PCR plate into the frame (position A1 goes to the upper left corner), ensuring that the plate fits accurately in the frame. Close the tray.
- Click “Start Run” to start.

Table 5: Standard PCR Program for the LightCycler® 480 Instrument.

Program Parameter	RT-Step	Cycling		Cooling
Analysis Mode	None	Quantification mode		None
Cycles	1	40		1
Segment	1	1	2	1
Target [°C]	63	92	60	40
Hold [hh:mm:ss]	00:20:00	00:00:05	00:00:40	00:00:10
Ramp Rate [°C/s]*	1.6	1.6	1.6	1.6
Acquisition Mode	None	None	Single	None

*

Select the detection format as created in section 8.1; activate the filter combinations for GAPDH, IGFBP3, F3 and VGLL3 and set the “Reaction Volume” to “20”.

The screenshot displays the software interface for configuring a PCR program. It is divided into three main sections: A, B, and C.

Section A: Programs
 This section shows a table of program parameters:

Program Name	Cycles	Analysis Mode
RT-step	1	None
cycling	40	Quantification
cooling	1	None

Section B: cycling Temperature Targets
 This section shows a table of cycling temperature targets:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
92	None	00:00:05	1,6		0	0	0
60	Single	00:00:40	1,6		0	0	0

Section C: cooling Temperature Targets
 This section shows a table of cooling temperature targets:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
40	None	00:00:10	1,6		0	0	0

Figure 3: Screenshot from LightCycler® 480 software v1.5 showing the run protocol of the Prostate® RT-qPCR Kit. A: Details of the RT-Step. B: Details of the Cycling Step. C: Details of the Cooling Step.

8.3 Analysis Settings on the LightCycler® 480 Instrument

8.3.1 General

For reliable quantification, the Crossing Point (“Cp”) of an amplification curve with the threshold shall be located in the early exponential phase of a PCR (exponential phase = straight segment of a PCR curve when displayed on a logarithmic scale).

It may be required to adjust the threshold level to guarantee reliable Cp values. To do so the horizontal threshold line in the Amplification Plot may be adjusted manually in compliance with the following rules (see Figure 4 below):

1. The threshold shall cross the PCR curves during the early exponential phase.
2. The threshold shall be located above the baseline of the PCR curves.
3. The threshold shall be within a defined upper/lower limit of the “STD Multiplier” as specified for each individual gene in section 8.3.2.

Each amplification signal should be visually inspected for irregular amplification. Signals crossing the threshold due to inconsistent data points (noise peaks) or linear curve shape should not be evaluated. In case of irregular amplification, the affected well must be omitted from the analysis by removing the “Include” checkmark in the Sample Table.

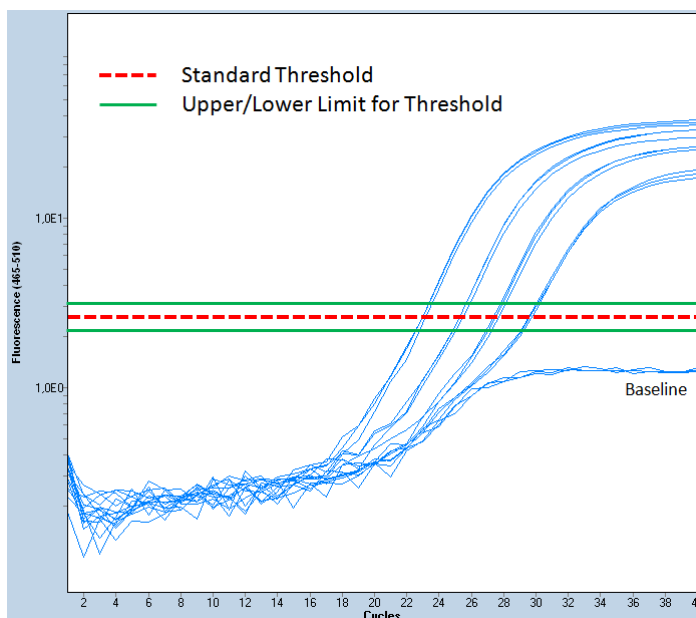


Figure 4: Screenshots of amplification curves using the LightCycler® 480 Instrument (logarithmic scale). The red dotted line shows the threshold using the standard “STD Multiplier” values as indicated in section 8.3.2. The threshold may be adjusted within the indicated upper and lower limit if required.

8.3.2 Analysis Procedure

NOTE: Perform data analysis only on used wells by generating a respective sample subset as described in the manual of the LightCycler® 480 Instrument.

- After the run has finished click “Analysis” on the LightCycler® 480 Basic Software Module bar opening the “Analysis Overview” window.
- Select “Abs Quant/Fit Points”.
- In the “Create new analysis” window choose the respective subset containing all used wells and confirm.
- Activate color compensation by clicking the arrow button next to “Color Comp (Off)” then click “In Use” and select the respective color compensation file.
- Set “First Cycle” to “1” and “Last Cycle” to “40”.

- Set the background (click blue “Background” button) to “6 - 11” by setting “Min Offset” to “5” and “Max Offset” to “10” in the “Cycle Range” window.

GAPDH:

- Activate the respective filter combination for GAPDH:
 - LightCycler® 480 I device: “Filter Comb 523 – 568”
 - LightCycler® 480 II device: “Filter Comb 533 – 580”
- Set the noise band to “Noise Band (STD Mult)” and set the “STD Multiplier” manually to “**50.0**” in the “Noise Band” window.
- If required, the threshold level may be adjusted manually in compliance with the rules in section 8.3.1. Ensure that the displayed “STD Multiplier” value is between **40.0** and **60.0**.
- Ensure the Threshold value in the “Analysis” window equals the Noise Band value.
- Ensure the number of Fit Points is “2”.
- Click “Calculate”.
- GAPDH Crossing Point (“Cp”) for each sample is displayed in the Sample Table.
- Export the Cp values by clicking with the right mouse button within the Sample Table. Choose “Export”. Save the file under the experiment name containing the gene name “GAPDH”.

IGFBP3:

- Activate the respective filter combination for IGFBP3:
 - LightCycler® 480 I device: “Filter Comb 558 – 610”
 - LightCycler® 480 II device: “Filter Comb 533 – 640”
- Keep all settings as adjusted before with one exception:
- Set the STD Multiplier manually to “**35.0**” in the “Noise Band” window.
- If required the threshold level may be adjusted manually in compliance with the rules in section 8.3.1. Ensure that the displayed “STD Multiplier” value is between **28.0** and **42.0**.
- Ensure the Threshold value in the “Analysis” window equals the Noise Band value.
- Ensure the number of Fit Points is “2”.
- Click “Calculate”.
- IGFBP3 Crossing Point (“Cp”) for each sample is displayed in the Sample Table.
- Export the Cp values by clicking with the right mouse button within the Sample Table. Choose “Export”. Save the file under the experiment name containing the gene name “IGFBP3”.

F3:

- Activate the respective filter combination for F3:
 - LightCycler® 480 I device: “Filter Comb 483 – 533”
 - LightCycler® 480 II device: “Filter Comb 465 – 510”
- Keep all settings as adjusted before with one exception:
- Set the STD Multiplier manually to “**80.0**” in the “Noise Band” window.
- If required, the threshold level may be adjusted manually in compliance with the rules in section 8.3.1. Ensure that the displayed “STD Multiplier” value is between **64.0** and **96.0**.
- Ensure the Threshold value in the “Analysis” window equals the Noise Band value.
- Ensure the number of Fit Points is “2”.

- Click “Calculate”.
- F3 Crossing Point (“Cp”) for each sample is displayed in the Sample Table.
- Export the Cp values by clicking with the right mouse button within the Sample Table. Choose “Export”. Save the file under the experiment name containing the gene name “F3”.

VGLL3:

- Activate the respective filter combination for VGLL3:
 - LightCycler® 480 I device: “Filter Comb 615 – 670”
 - LightCycler® 480 II device: “Filter Comb 618 – 660”
- Keep all settings as adjusted before with one exception:
- Set the STD Multiplier manually to “**27.5**” in the “Noise Band” window.
- If required the threshold level may be adjusted manually in compliance with the rules in section 8.3.1. Ensure that the displayed “STD Multiplier” value is between **22.0** and **33.0**.
- Ensure the Threshold value in the “Analysis” window equals the Noise Band value.
- Ensure the number of Fit Points is “2”.
- Click “Calculate”.
- VGLL3 Crossing Point (“Cp”) for each sample is displayed in the Sample Table.
- Export the Cp values by clicking with the right mouse button within the Sample Table. Choose “Export”. Save the file under the experiment name containing the gene name “VGLL3”.

8.4 Analysis Settings on other verified Instruments

In the CFX96 Touch, Quant Studio 5, & 7500 Fast instrument data analysis can be performed by adjusting the baseline while the threshold can be kept automatic as below:

- Baseline: “User defined” 6-11
- Threshold: automatic from software

Note: It is recommended to use the threshold automatically calculated by the instrument analysis software. It is possible to adjust the threshold manually to meet the validity criteria of Prostatype® RT-qPCR Kit for the positive controls.

In this phase of analysis, the user should focus on the Positive Control signal to ensure the run meets the acceptance and validity criteria.

8.5 Validity of Prostatype® RT-qPCR Runs

The Prostatype® RT-qPCR run is considered valid if criteria set forth for the Prostatype Positive Control and the Prostatype Negative Control in the following tables are met.

If either the Prostatype Positive Control or Prostatype Negative Control (or both) is invalid, the data for patient samples cannot be interpreted. Testing must be repeated for all patient samples included in this run.

NOTE: Use the median Cp values/ Δ Cp values out of the three replicates received for each sample for the following calculations. Median refers to the value in the middle when arranging all values from the lowest to the highest (see example in Table 10 on page 20). When only two values are available the mean/average of those two values is used. For either the controls or patient samples three PCR replicates must be performed and evaluated. In case of an irregular curve, one of the three results might be omitted, such that at least two replicates remain for evaluation.

8.5.1 Evaluation of the Prostatype Positive Control

- Check if results of at least two PCR replicates of the Positive Control are available.
- If no Cp value is reported for a gene use the number “38” as a “simulated Cp” in order to calculate a Δ Cp value.
- Identify the median Cp value for GAPDH.
- Check if the validity limit for GAPDH is met:

Table 6: GAPDH Cp Validity Limit of the Prostatype Positive Control.

Gene	Cp Validity Limit
GAPDH	$20.00 \leq \text{Median Cp} \leq 22.00$

- Calculate the Δ Cp values for IGFBP3, F3 and VGLL3 for every well according to the following formula:

$$\Delta Cp [gene] = Cp\ value [gene] - Cp\ value [GAPDH]$$

(Wherein gene is IGFBP3, F3 or VGLL3)

- Identify the median Δ Cp values for IGFBP3, F3, and VGLL3.
- Check if the validity limits for IGFBP3, F3, and VGLL3 are met:

Table 7: Δ Cp Validity Limits of the Prostatype Positive Control.

Gene	Δ Cp Validity Limits
IGFBP3	$1.90 \leq \text{Median } \Delta Cp \leq 3.90$
F3	$0.90 \leq \text{Median } \Delta Cp \leq 2.90$
VGLL3	$2.80 \leq \text{Median } \Delta Cp \leq 4.80$

8.5.2 Evaluation of the Prostatype Negative Control

- Check if results of at least two PCR replicates of the Negative Control are available
- Identify the median Cp value for GAPDH.
- Check if the validity limits for the Prostatype Negative Control are met:

Table 8: Validity Limits of the Prostatype Negative Control.

Gene	Cp Validity Limits
GAPDH	$29.00 \leq \text{Median Cp} \leq 31.00$
IGFBP3, F3, VGLL3	no Cp

8.6 Validity of Individual Patient Sample Wells

Only if the external controls are valid according to section 8.5, the results of the internal control for each patient sample well can be evaluated. An individual well is valid, if the criterion for the internal control set forth in Table 9 is met.

- Check if the validity limit for individual patient sample wells is met:

Table 9: Validity Limit of Patient Samples.

Gene	Cp Validity Limit
GAPDH	Median Cp \leq 28.00

- Ensure that at least two patient sample wells meet this criterion.

If a patient sample well exceeds the validity limit, the data for that patient sample well cannot be interpreted. At least two valid patient sample wells are required for further data analysis. Otherwise, the patient sample result is invalid and testing of the patient sample must be repeated.

9 Data Analysis for Patient Samples

If all requirements outlined in section 8.4 and 8.5 are met, the results of an individual sample can be calculated and reported. The mRNA expression levels of the three genes IGFBP3, F3, and VGLL3, relative to the expression level of gene GAPDH will be indicated by the median Δ Cp determined as outlined below. Table 10 shows an example of how to calculate the Median Δ Cp for one gene.

- Calculate the Δ Cp values for IGFBP3, F3, and VGLL3 for every well according to the following formula:

$$\Delta Cp [gene] = Cp \text{ value } [gene] - Cp \text{ value } [GAPDH]$$

(Wherein gene is IGFBP3, F3 or VGLL3)

- If no Cp value is reported the gene was expressed below the detection limit. In order to calculate a Δ Cp, use the number “38” as a “simulated Cp”.
- Identify the Median Δ Cp values for IGFBP3, F3, and VGLL3.

Table 10: Example for the calculation of the Median Δ Cp for VGLL3 for one sample. A similar calculation must be performed for the genes F3 and IGFBP3.

PCR Replicate	Cp Value GAPDH	Cp Value VGLL3	Calculated Δ Cp	Median Δ Cp VGLL3
Well 1	20.25	25.36	$\Delta Cp_1 = 25.36 - 20.25 = 5.11$	Median {5.11; 4.99; 18.02} = 5.11
Well 2	20.15	25.14	$\Delta Cp_2 = 25.14 - 20.15 = 4.99$	
Well 3	19.98	no Cp	$\Delta Cp_3 = 38 - 19.98 = 18.02$	

10 Quality Control

10.1 External Controls

The Prostatype® RT-qPCR Kit contains Prostatype Positive Control and Prostatype Negative Control. These controls must be included in triplicate in each individual PCR run to ensure validity of test results. The Prostatype

Positive Control and Negative Control values must be within the validity limits (see Table 6, or Table 7, or Table 8). If a control is out of its specified range, the associated test results are invalid, must not be reported and testing must be repeated.

10.2 Internal Controls

For every patient sample, the expression level of the reference gene GAPDH is determined and used to evaluate the sample validity and integrity. The GAPDH level must be above a specific value to guarantee a robust measurement of the target genes (see Table 9). Furthermore, the internal control is used to verify that enough amplifiable RNA was extracted during the RNA extraction step.

11 Limitations of the Procedure

The product shall be used by personnel experienced in molecular biological techniques, specifically real-time PCR. General recommendations on the organization and procedures of the laboratory shall be obeyed in order to prevent DNA contamination.

All results from Prostatype® RT-qPCR Kit must be evaluated in conjunction with patient clinical history and other available information.

12 Performance of the Prostatype® RT-qPCR Kit

The Prostatype® RT-qPCR Kit has been validated to be able to generate Cp (GAPDH) ≤ 28 with around 95 % possibility by using specimen collected to the following criteria:

- Unstained FFPE tissue sections from core needle biopsies were used.
- Sections were of 10 μm thickness.
- The minimum tumor area used was 30 mm^2 with tumor content $>50\%$.

For samples with a tumor area between 15 mm^2 and 30 mm^2 and 50% cancer cells, it is still encouraging to continue performing RNA extraction and Prostatype® RT-qPCR test, since there is $>90\%$ possibility to obtain Cp(GAPDH) ≤ 28 from these specimens. For specimens with a tumor area $<15\text{mm}^2$ and 50% cancer cells, there is around 80% possibility to generate Cp(GAPDH) ≤ 28 . Samples with $<5\text{mm}^2$ tumor area are not suitable for testing with Prostatype® RT-qPCR Kit.

12.1 RNA Input

Compatibility of the Prostatype® RT-qPCR Kit with use of total RNA extracted from FFPE tissue from human prostate core needle biopsy as input was validated by assessment of 16 individual clinical specimens. Total RNA was extracted from unstained prostate core needle biopsies containing at least 50% cancer cells. The specimen collection was done according to the following criteria: area $\geq 30 \text{ mm}^2$, thickness 10 μm . RNA extraction kit as recommended in section 7 was used. Results of Prostatype® RT-qPCR Kit were valid for around 95 % of the specimen.

12.2 Linearity/ Precision/Limit of Quantitation

Linearity was evaluated by testing a dilution series of synthetic target RNA covering a range from 2.5×10^6 to 38 molecules/ μL . Testing comprised three different Prostatype® RT-qPCR Kit Lots. For GAPDH and F3 assay the linear range covers the concentrations from 2.5×10^6 to 38 copies/ μL . For IGF3P3 linearity is given down to 153 copies/ μL , and for VGLL3 down to 305 copies/ μL . Within the reported range, the assays present linearity with a regression coefficient $R^2 > 0.99$ (see Figure 5). For the linear range, slopes have been observed between -3.12 and -3.29. Standard deviation (SD) and coefficient of variation (CV) were calculated within the defined

range of linearity. The largest SDs of 0.4 Cp were detected for IGFBP3 at a concentration of 153 copies/ μ L and for VGLL3 at a concentration of 305 copies/ μ L. The largest CV was 1.6 % for VGLL3 at a concentration of 3.9×10^4 copies/ μ L. The Limit of Quantitation of the Prostatype® RT-qPCR is represented by the lower level of the linear range of the respective assay stated above.

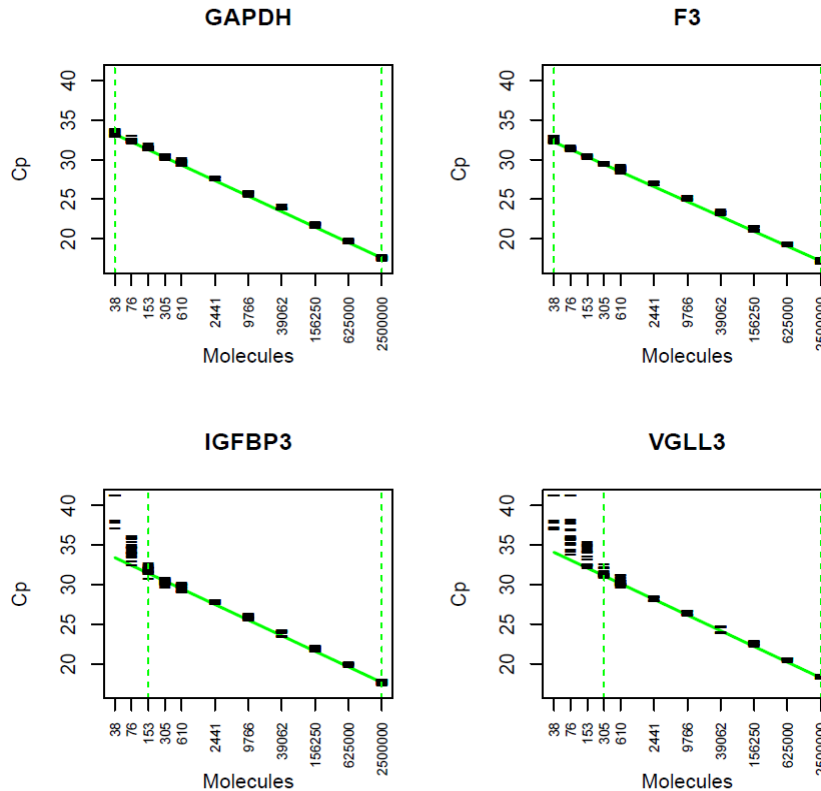


Figure 5: Linearity of the four genes contained in the Prostatype® RT-qPCR. The observed Cp values (black lines) are plotted for the 11 tested target concentrations ranging from 38 to 2.5×10^6 molecules per μ L. Each target concentration was assessed by at least 15 PCR replicates. The fitted linear regression is shown as a solid green line.

12.3 Limit of Detection

The limit of detection was evaluated by repeated analysis of synthetic target RNA covering a range from 2.5×10^6 to 38 molecules/ μ L. The four lowest target concentrations were run in 24 replicates involving three different Prostatype® RT-qPCR Kit lots. The lowest concentrations showing amplification curves for all replicates (100% detection) were as follows: GAPDH: 38 copies/ μ L, F3: 38 copies/ μ L, IGFBP3: 76 copies/ μ L, VGLL3: 153 copies/ μ L.

12.4 Relative Sensitivity

Relative sensitivity was evaluated by testing samples containing different proportions of IGFBP3, F3, and VGLL3 target in an equal amount of GAPDH background. Following samples were tested in 6 replicates per sample with different Prostatype® RT-qPCR Kit lots: 800 copies/ μ L, 400 copies/ μ L and 200 copies/ μ L of IGFBP3, F3, and VGLL3 target in an equal amount of 4.0×10^4 copies/ μ L GAPDH background resulting in proportions of 1:50, 1:100 and 1:200. For each of the four assays, all replicates of the samples (18 PCR wells)

showed an amplification curve (100% detection), confirming a relative sensitivity for the three target assays below 1%.

12.5 Limit of Blank

Twenty-one (21) PCR wells containing no RNA targets were tested with different Prostatype® RT-qPCR Kit lots to evaluate the limit of blank. The three target genes (IGFBP3, F3, and VGLL3) showed no amplification curve for all 21 wells. Therefore, the limit of blank corresponds to the maximal observable Cp value of 38 for those genes. For GAPDH 16 of the 21 PCR wells showed an amplification curve with Cp values between 35.2 and 38.0. The limit of blank for GAPDH was estimated by the 95% quantile and equals Cp 35.2.

12.6 Reproducibility

Reproducibility was evaluated by repeated testing of aliquots from eight RNA pools extracted from FFPE human prostate core needle biopsy containing cancer cells. Testing was performed by four operators at three different testing laboratories across Europe. Each testing site prepared 12 independent PCR plates involving three different Prostatype® RT-qPCR Kit lots. Results of the evaluation are detailed in Table 11.

Table 11: Results of reproducibility testing. Mean Cp and related standard deviation (SD) and coefficient of variation (CV) are listed for the four genes. All eight RNA pools were measured in 36 independent PCR runs.

RNA Sample	GAPDH			F3			VGLL3			IGFBP3		
	Mean Cp	SD (Cp)	CV (%)	Mean Cp	SD (Cp)	CV (%)	Mean Cp	SD (Cp)	CV (%)	Mean Cp	SD (Cp)	CV (%)
Pool 1	23.9	0.150	0.626	26.2	0.295	1.126	26.4	0.316	1.198	27.2	0.327	1.205
Pool 2	25.4	0.189	0.742	27.6	0.302	1.096	28.4	0.388	1.366	27.6	0.315	1.140
Pool 3	24.4	0.208	0.856	26.2	0.307	1.172	28.9	0.474	1.639	26.5	0.292	1.100
Pool 4	24.6	0.267	1.086	25.6	0.288	1.122	28.1	0.459	1.632	26.5	0.299	1.131
Pool 5	23.4	0.203	0.867	25.3	0.300	1.184	27.1	0.357	1.318	25.4	0.258	1.019
Pool 6	24.2	0.181	0.749	26.5	0.299	1.126	29.5	0.609	1.060	26.4	0.265	1.002
Pool 7	24.0	0.165	0.685	26.2	0.277	1.059	28.8	0.457	1.586	26.1	0.297	1.141
Pool 8	23.6	0.186	0.791	25.1	0.228	0.909	28.2	0.408	1.447	25.7	0.296	1.154

12.7 Interference

Aliquots from three RNA pools unspiked and spiked with potentially interfering substances were processed. No interference was observed with elevated levels of Hematoxylin/Eosin (HE, 0.01% v/v), Xylol/Xylene (0.01% v/v), DNase I (0.06 U/μL), Human genomic DNA (20 ng/μL), Guanidinium Chloride (5 mM), Ethanol (1% v/v) and EDTA (1mM).

13 Precautions and Safety

To protect yourself as well as to avoid contamination of reagents and samples, always wear a laboratory coat and disposable gloves when using the Prostatype® RT-qPCR kit. This measure is also critical when scraping cancer tissue for RNA extraction.

NOTE: The Prostatype® RT-qPCR Kit does not contain any hazardous ingredients. The product does not contain any infectious substances or agents, which cause disease in humans or animals.

NOTE: The respective material safety data sheets (MSDS) are available on our website and provide more detailed information (www.prostatypegenomics.com/technical-documents).

To obtain reliable results, it is *imperative* to adhere to the instructions given in the IFU. Deviations from these guidelines may cause the generation of incorrect or invalid data or may lead to the failure to obtain test results.

General:

- The Prostatype RT-qPCR kit is for use in a professional laboratory and only be used by professional laboratory personnel that has been trained and certified by Prostatype Genomics according to the corresponding training document.
- Avoid microbial contamination of reagents when removing aliquots from reagent bottles.
- To prevent contamination by amplicons generated from previous PCR reactions, a strict separation of pre-PCR activities (e.g. RNA extraction, PCR setup) and PCR activities (e.g. real-time PCR) is strongly recommended. Preferably, a working area exclusively used for RNA testing shall be used.
- General recommendation on the organization and procedures of the laboratory shall be adhered to in order to prevent DNA contamination. Always wipe all working areas, surfaces, equipment and materials with a DNA decontamination agent and an RNase decontamination agent before starting the procedure.

Patient Sample handling and RNA extraction

- Samples intended for analysis with the Prostatype RT- qPCR kit are to be selected by a professional pathologist or a professional with equivalent training.
- An optimal amount of 30 mm² tissue containing at least 50% cancer cells is recommended for each patient sample.
- Good laboratory practice is essential to minimize the risk of cross-contamination between samples during and after RNA extraction and purification.
- The use of sterile pipette tips with an aerosol barrier is recommended to prevent cross-contamination of patient samples.

PCR activities

- Do not store RNA after extraction and ensure that freshly extracted RNA is used in the Prostatype® RT-qPCR test within one day.
- Preparation of the Prostatype® RT-qPCR shall be performed at room temperature (15-25 °C).
- We further recommend that used PCR plates should be placed in a re-sealable plastic bag or equivalent immediately after removal from the PCR instrument. The bag shall be closed and disposed of in a dedicated waste container. Never store a used PCR plate outside of the PCR instrument. Never open a used PCR plate.

14 Meaning of Symbols



Consult Instructions for Use



Catalogue Number



European conformity



In Vitro Diagnostic Medical Device



Lot number



Expiration Date



Manufacturer



Temperature Limitation



Contains sufficient for <n> tests

15 References

1. Peng Z, Skoog L, Hellborg H, Jonstam G, Wingmo IL, et al. (2014) An expression signature at diagnosis to estimate prostate cancer patients' overall survival. *Prostate Cancer Prostatic Dis* 17:81-90.
2. Fabian S, Li-Di X, Johan B, Michael H et al. A Novel Risk Score (P-score) Based on a Three-Gene Signature, for Estimating the Risk of Prostate Cancer-Specific Mortality. *Research and Reports in Urology* 2022:14 203–217.
3. Peng Z, Andersson K, Lindholm J, Bodin I, Pramana S, et al. (2014) Operator dependent choice of prostate cancer biopsy has limited impact on a gene signature analysis for the highly expressed genes IGFBP3 and F3 in prostate cancer epithelial cells. *PLoS One* 9: e109610.
4. Peng Z, Andersson K, Lindholm J, Dethlefsen O, Pramana S, et al (2016) Improving the prediction of prostate cancer overall survival by supplementing readily available clinical data with gene expression levels of IGFBP3 and F3 in formalin-fixed paraffin embedded core needle biopsy material. *PLoS One* 11: e0145545.
5. PWS IFU, Prostatype Genomics.

16 Contact Information

The **Prostatype® RT-qPCR Kit** is manufactured by:

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