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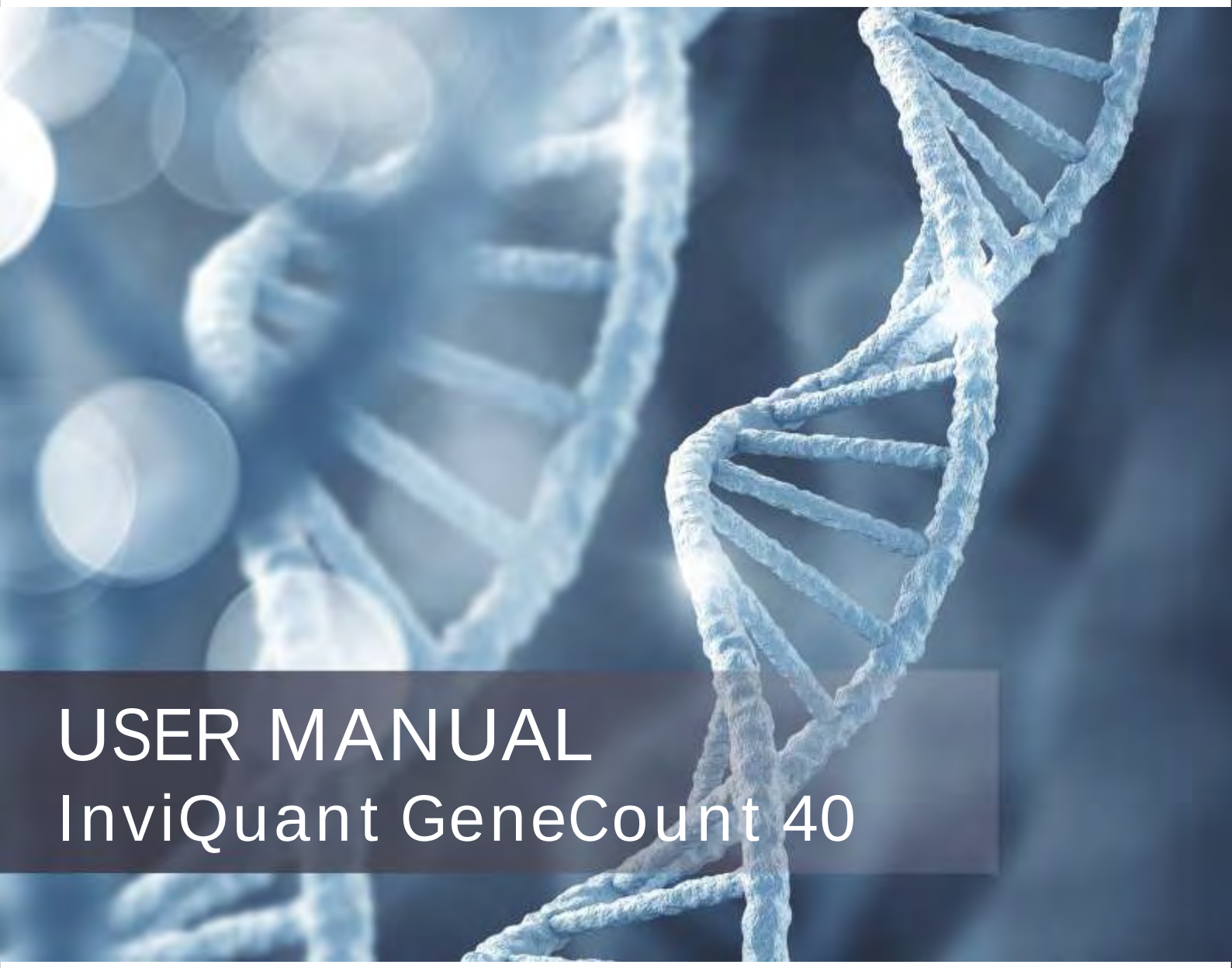

USER MANUAL
InviQuant GeneCount 40

real-time PCR-System for the detection and quantification of human DNA

REF 3130100100

Invitek Molecular GmbH · D-13125 Berlin

Instruction for InviQuant GeneCount 40

InviQuant GeneCount 40 is a real-time PCR-system for the detection and quantification of human genomic DNA. By choosing a human specific multi-copy target very small amounts of human DNA can be detected and quantified, especially from challenging samples such as cell-free circulating DNA (cfDNA), formalin-fixed paraffin-embedded (FFPE) tissue and forensic samples.
The **InviQuant GeneCount 40** system uses a qPCR assay to query 40 discrete genomic loci that are randomly distributed in the genome for the quantification and qualification of DNA samples. This proprietary design ensures minimal variation caused by local genome events, like copy number changes or SNPs.

This real-time PCR detection method should only be performed by laboratory personnel trained in molecular biology methods.

Content

Kit contents of the InviQuant GeneCount 40

Kit Code	Reagent	Amount for 200 reactions	Lid Color
1	Reaction Mix	3 x 1500 µl	yellow
2	Taq Polymerase	1 x 22 µl	red
3	Positive Control	1 x 250 µl	light blue
4	Dilution Buffer	2 x 2000 µl	white
5	Standard DNA	1 x 250 µl	dark blue

Symbols


Manufacturer

Lot number

Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse

Attention: *Do not combine components of different kits, unless the lot numbers are identical!*

Storage

Protect all reagents of the **InviQuant GeneCount 40** from light and store at -20 °C.

- All reagents can be used until the expiration date, but have a minimum shelf life of 12 month
- After expiry, the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 – 8 °C).
- During PCR preparation, all the reagents should be stored on ice.

Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviQuant GeneCount 40** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular identifies a problem in the lot, Invitek Molecular will replace the product free of charge.
Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **InviQuant GeneCount 40** is tested against predetermined specifications to ensure consistent product quality.
In case of any questions or problems regarding any aspects of **InviQuant GeneCount 40** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request and are presented at the Invitek Molecular webpage www.invitek-molecular.com.
For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviQuant GeneCount 40** assay is a real-time PCR-System to determine quantity and quality of human genomic DNA. The system has been developed for discovery, translational, and clinical researchers who need a cost-effective way to determine the optimum conditions for targeted enrichment of DNA isolated from biological samples, especially those preserved as formalin-fixed, paraffin-embedded (FFPE) samples, cfDNA and forensic samples.
This assay can be used for validating DNA samples e.g. if they are suited for amplification by targeted enrichment prior to NGS.
cfDNA plays a more and more important role in the field of “Liquid Biopsy” and moreover, is an invaluable sample source for the approach of “Personalized Medicine”. This DNA species is, as well as FFPE samples, damaged and fragmented (< 500 bp) to varying extents.
FFPE tissue archives are an invaluable source for the molecular characterization of disease using Next-Generation Sequencing (NGS). Unfortunately, genomic DNA present in FFPE samples is damaged and fragmented to varying extents depending on fixation and storage conditions. Because of this damage, only a fraction of the DNA may be usable as template for PCR.
Commonly used DNA quantification methods, including mass measurements by spectrometry or fluorescence, do not differentiate between amplifiable and non-amplifiable DNA. Therefore, they cannot reliably measure the amplifiable amounts of DNA that are able, e.g., to participate in the multiplex PCR-based targeted enrichment step in the NGS workflow. To overcome this challenge, the **InviQuant GeneCount 40** system utilizes a simple qPCR protocol to determine the amplifiable fraction of DNA present in a sample, and provide guidance for the appropriate amount of input for downstream applications, e.g. NGS.
The **InviQuant GeneCount 40** system can be integrated into any NGS workflow.
This method can be applied to a variety of human gDNA samples (including sorted cells, blood, free circulating or sonicated gDNA, etc.).

THE PRODUCT IS INTENDED FOR MOLECULAR BIOLOGY APPLICATIONS CARRIED OUT BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGY TECHNIQUES. THE **InviQuant GeneCount 40** IS NOT INTENDED FOR THE DIAGNOSIS, PREVENTION OR TREATMENT OF A DISEASE.

The Purchaser agrees that the use of this product and data therefrom is limited solely to the purchaser and for only the purchaser’s own internal molecular biology research applications (“Permitted Use”), and shall not be re-sold or used for any other purposes (all of which are expressly prohibited), including without limitation diagnostic purposes, uses that could require regulatory approval for diagnostics from an agency of any government or regulatory entity anywhere in the world, diagnosis, prevention, or treatment of disease, and the right to perform commercial services of any kind, including without limitation, reporting the results of purchaser’s activities, including without limitation, for a fee or other commercial consideration. Except for the Permitted Use, no rights, titles, or interests in or to any tangible or intangible property rights are conveyed or shall be deemed conveyed by implication, estoppel or otherwise. The performance characteristics of the product other than for the Permitted Use are unknown.

Product use limitation

The **InviQuant GeneCount 40** assay only is suitable for the measurement of human genomic DNA.
The presence of PCR inhibitors may cause invalid results.
Mutations or polymorphisms in primer or probe binding regions affect the detection resulting in a false negative result with the **InviQuant GeneCount 40**.
As with all PCR based tests, extremely low levels of target below the limit of reliable detection may be detected, but results may not be reproducible.
The included chemicals are only useable once.
The user is responsible to validate the performance of the Invitek Molecular product for any particular use.
Invitek Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All products sold by Invitek Molecular are subject to extensive quality control procedures (according to EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection thereof.
The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.
The product with its contents is unfit for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!
Avoid skin contact! Adhere to the legal requirements for working with biological material!

- This test should only be performed by laboratory personnel trained in molecular biology methods.
- Physically separate the workspace for extraction, PCR preparation, PCR run and post-PCR work to avoid contaminations.
- Strictly follow the working instructions.
- Before setting up an experiment, decontaminate the PCR workspace and labware (pipet barrels, tube racks, etc.) with 5% bleach and UV light. Preferentially set up reactions in a PCR workstation.
- Wear gloves throughout the entire procedure.
- Close all tubes containing PCR products as soon as possible after use.
- Do not smoke, eat or drink in areas where samples are handled.
- Do not use this product after the expiration date.

The **InviQuant GeneCount 40** does not require any European Community risk and safety phrases for its components.

Product characteristics of the InviQuant GeneCount 40

Starting material	Time for PCR run	Limit of Detection
5 µl DNA sample (total PCR reaction volume: 25 µl)	< 30 min	≥ 5 copies

Unlike other approaches that target a single genomic locus, the **InviQuant GeneCount 40** system uses a qPCR assay to query 40 discrete genomic loci that are randomly distributed in the genome for the quantification and qualification of DNA samples. This proprietary design ensures minimal variation caused by local genome events, like copy number changes or SNPs. The assay is performed using a real-time PCR instrument (see overview at page 13). The amplified target sequence is detected using hydrolysis probes. The probes are labeled at one end with a quencher and at the other end with a reporter fluorescent dye (fluorophore). In the presence of the target sequence the probe hybridizes to the amplicon and the quencher is removed from the probe via the 5´-3´ exonuclease activity of the polymerase, thus enabling the dye to fluoresce when excited by its specific wavelength.
The test contains an internal amplification control for the determination of PCR inhibitors, as well as a standard DNA for preparing a dilution series with defined copy numbers. The use of a Standard DNA dilutions series (see Data analysis, page 11) allows quantification of amplifiable molecules in gDNA samples. Therefore, the **InviQuant GeneCount 40** assay is highly suited for determining the correct input amount of DNA in downstream experiments (e.g. the multiplex PCR-based targeted enrichment step in NGS workflows) in particular from FFPE, cfDNA and forensic samples.
This very sensitive assay can even detect minimal DNA contaminations (e.g. human DNA traces from the executive personnel). This can lead to positive signals in the negative controls. Therefore, no qualitative statements can be made at a Ct-value difference ≤ 1.5 between the Ct value of the negative control and the Ct-value of the sample DNA.
The workflow for the **InviQuant GeneCount 40** assay is simple and convenient. After extracting gDNA from biological samples, simply add the DNA to the qPCR Master-Mix. Real-time PCR is performed according to manufacturer's recommendations and Ct-values are exported to calculate the concentration of the sample by using the linear equation generated with the Standard DNA dilutions series.

Principle and procedure

The Master-Mix is prepared and aliquoted into appropriate tubes or wells. Genomic DNA extracted from biological samples is added to the Master-Mix. PCR is performed and the raw Ct-values are used to quantify and qualify the sample DNA.

Note: *Optimal results are obtained when DNA sample concentration is between 100 ng/µl and 0.2 pg/µl. DNA extracted using methods and samples that yield high amounts of DNA might need to be diluted first. If you are using a new method to extract DNA, serial dilutions of the DNA sample are recommended to ensure at least one concentration is within the range and to test if any PCR inhibitors are present in the DNA sample.*

Note: *Always include the Standard DNA dilutions series (see Data analysis, page 11) into every run, regardless of the number of gDNA samples processed. Calculations cannot be performed in the absence of the Standard DNA dilutions deries. For economic use of reagents it is recommended to test at least 16 DNA samples per dilution series.*

Important notes

Important points before starting a PCR run

Immediately upon receipt, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities please notify Invitek Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitek Molecular Technical Services or your local distributor.

- Do not use damaged kit components, since their use may lead to poor kit performance.
- Always change pipette tips between liquid transfers.
- To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of cross contamination, we recommend working in a PCR workstation.
- This kit should only be used by trained personnel.

Important tips

1. Prepare Standard DNA dilution series and Master Mix always fresh.
2. Please note that NTC, PTC and DNA dilution series reduces the number of actual PCR reactions. Thus, it is recommended to test at least 16 DNA samples per PCR run.
3. Optimal results are obtained when DNA sample concentration is between 100 ng/µl and 0.2 pg/µl.
4. For optimal results, it is recommended to prepare the Standard DNA dilution series in triplicates and NTC and PTC in duplicates. DNA samples are measured in single reactions.

Reagents and equipment to be supplied by user

- Real-time PCR instrument, equipped with two detection channels 510 nm and 580 nm
- Real-time PCR consumables (plates, tubes, foils, caps)
- Pipettes with filter tips
- Vortexer
- Powder-free disposal gloves
- Microcentrifuge with a rotor for the reaction tubes

For more information regarding sample storage, including forensic samples please refer to Appendix I

Invitek Molecular will be released of its responsibilities if other material than purified DNA as described in the Intended Use are processed or if protocols are changed or modified.
The **InviQuant GeneCount 40** is optimized for the detection and quantification of human DNA extracted using the following Kits: **see** Appendix II

Product limitations

The **InviQuant GeneCount 40** real-time PCR-System has a limit of detection of ≤ 5 DNA copies. The assay limit of detection depends on sample matrix, DNA preparation and DNA content. The PCR reaction is inhibited at DNA concentrations of > 100 ng/µl.

Protocol 1: Preparing the Standard DNA Dilution Series

For the preparation of the Standard DNA Dilutions Series dilute the Standard DNA (Code 5, dark blue lid) in 1:10 steps in Dilution Buffer (Code 4, white lid). A total of 4 dilutions (S1 to S4) is required.

1. Label 1 test tube (not provided) with `Premix´, add 45 µl of Dilution Buffer (Code 4, white lid) and 5 µl Standard DNA (Code 5, dark blue lid). Vortex thoroughly and spin down.
2. Label 4 test tubes (not provided) S1 to S4 and fill every test tube with 45 µl Dilution Buffer.
3. Prepare the serial dilution after the following table.

Pipetting scheme for the preparation of the dilution series

Standard	Dilution	Copies/ µl	Copies/ Reaction *	Genomic equivalents/ Reaction**	ng DNA/Reaction***	ng DNA/µl****
S1	45 µl Dilution Buffer + 5 µl Premix (vortex thoroughly and spin down)	10,000	50,000	1,250	7.5	1.5
S2	45 µl Dilution Buffer + 5 µl S1 (vortex thoroughly and spin down)	1,000	5,000	125	0.75	0.15
S3	45 µl Dilution Buffer + 5 µl S2 (vortex thoroughly and spin down)	100	500	12.5	0.075	0.015
S4	45 µl Dilution Buffer + 5 µl S3 (vortex thoroughly and spin down)	10	50	1.25	0.0075	0.0015

* 5 ul Standard DNA are used per reaction. The total number of copies per reaction has to be recorded in the setup file of the software program of the real-time PCR instrument.
** Calculation: Copies per reaction / 40
*** Calculation: Genomic equivalents per reaction*0.006 ng (1 diploid human cell contains 6 pg (= 0.006 ng) of DNA)
**** Calculation: ng DNA per reaction / 5

Note: *For economic use of reagents, it is recommended to test at least 16 DNA samples per dilution series.*

Internal control

The provided Reaction Mix contains an internal amplification control (IAC) that serves as inhibition control.

Protocol 2: Preparing the Master-Mix

1. Calculate the total number of required PCR reactions (sample and control reactions) and add an extra 10% to compensate for pipetting errors.
2. Thaw, vortex and centrifuge reagents before use, except the Taq Polymerase.
3. The Taq Polymerase should not be vortexed.
4. The following controls are recommended: Positive Control (Code 3, light blue lid), Negative Control.
5. For quantification, a Standard DNA is provided to prepare a dilution series (see page 8).

Example for the calculation and preparation of 10 reactions:

Component	1 Reaction	10 Reactions (incl. 10%)
Reaction Mix	19.9 µl	218.9 µl
Taq Polymerase	0.1 µl	1.1 µl
Total volume	**20 µl**	**220 µl**

After preparation of the Master-Mix vortex 5 sec and centrifuge briefly.

Protocol 3: Preparing the PCR-Mix

***Note*:** *Optimal results are obtained when DNA sample concentration is between 100 ng/µl and 0.2 pg/µl. DNA extracted using methods and samples that yield high amounts of DNA might need to be diluted first. If you are using a new method to extract DNA, serial dilutions of the DNA sample are recommended to ensure at least one concentration is within the range and to test if any PCR inhibitors are present in the DNA sample.*

1. Pipet 20 µl of master mix into each appropriate tubes or wells.
2. For the Negative Control pipet 5 µl of Dilution Buffer (Code 4, white lid) into the designated PCR tubes or wells and close them immediately. For reliable results it is recommended to prepare the Negative Control in duplicates.
3. Pipet 5 µl of sample DNA into the designated tube or well. Close immediately.
4. Pipet 5 µl of the Positive Control (Code 3, light blue lid) into the designated tubes or wells. Close immediately. For reliable results it is recommended to prepare the Positive Control in duplicates.
5. Pipet 5 µl of each standard DNA dilutions (S1 to S4) into the designated tubes or wells. Close immediately. For an accurate standard curve it is recommended to prepare the standard DNA dilution in triplicates.
6. Centrifuge all tubes/plate shortly at low speed.
7. Place tubes/plate into the PCR instrument and start the run according to the setup.

Setup

Step	Rotorcycler / LightCycler® 480 II / Cobas z	Blockcycler / Cepheid SmartCycler®
Initial Denaturation (HOLD) Cycles Denaturation Annealing/Extension (CYCLE)	1 min, 95 °C 45 10 sec, 95 °C 15 sec, 60 °C	1 min, 95 °C 45 15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate/ Ramp Rate	Maximum	Maximum

Data Analysis

The evaluation has to be made according to the analysis program recommended by the real-time PCR instrument manufacturer. Using the Log View of the amplification plot, place the threshold above the background signal but within the lower third of the linear portion of the amplification curves. The control reactions need to give the correct results.

- If there are contaminations by the target DNA, the Negative Controls can show positive signals.
- The Positive Control should be detected at Ct-values between 26 and 30 depending on the PCR instrument used (Rotorcycler: Ct 26-28; Blockcycler: Ct 28-30).
- A sample is stated **positive** (measurable), if the sample DNA shows amplification in the FAM-channel. The Ct-value difference between the Negative Controls and the sample DNA should be ≥ 1.5 cycles.
- A sample is stated **negative** (no measurable human DNA), if the sample DNA shows no amplification in the FAM-channel, the Ct-value difference between the Negative Controls and the sample DNA is ≤ 1.5 cycles and the internal amplification control (VIC/HEX-channel) of the sample is **positive**.
- If the sample DNA and the internal amplification control are **negative** the sample contains PCR-inhibiting substances or the sample preparation was not successful. Under these circumstances an evaluation of the samples is not possible. DNA isolation and purification for the sample need to be improved.

Note: *The IAC amplification curve (VIC/HEX-channel) gives a much lower fluorescence compared to the FAM channel. To evaluate the IAC please zoom into the lower portion of the amplification plot to check for amplification. The IAC amplification is for inhibition check only; it is not used for quantification.*

Concentration calculation

InviQuant GeneCount 40 contains a Standard DNA. For DNA quantification a dilution series (see chapter: Preparing the Standard DNA Dilution Series) has to be included in every run. Plot the Ct-values (y-axis) of the dilution series against copies/µl (x-axis) and create a logarythmic equation $y = m \times logx + b$. Note: concentrations on the x-axis are plotted logarithmically. Obtain the Ct-value of the DNA samples and calculate the concentration of your sample using the equation. For accurate results, the R-value of the equation should be ≥ 0.99.

Example

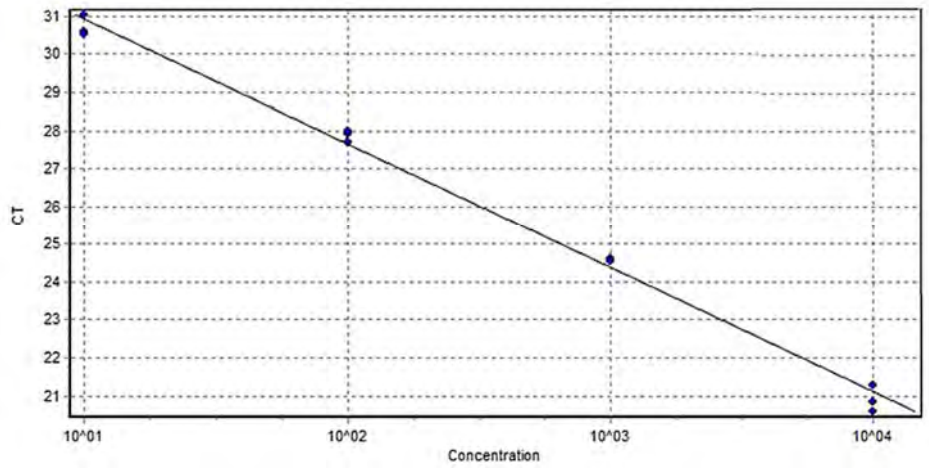

Figure 1: Example of the DNA dilution series in a semi-log plot. The Ct-value of each triplicate of the dilution series is plotted against its known concentration (copies/µl) and the equation $y = m \times logx + b$ is obtained.

Exemplary sample calculation

Formula: $y = m \times logx + b$

y = Ct-value of a measured sample
m = slope of the logarythmic equation
x = concentration (in copies/µl)
b = y-axis-intercept (0) = virtual CT of one unit (here 1 copy/µl)

Change the equation to x resulting in:

$$x = 10^{\left(\frac{y-b}{m}\right)}$$

Calculation with example values for the equation:

Assuming, the standard curve gives the values $m = -3.269$ and $b = 34.191$.

Assuming, a sample gives a Ct-value = **28**, the concentration is calculated as follows using the formula:

$$x = 10^{\left(\frac{\mathbf{28}-34.191}{-3.269}\right)}$$

- Calculation of copy numbers:

  $$x = 10^{\left(\frac{\mathbf{28}-34.191}{-3.269}\right)} = 78.32\ copies/\mu l$$

- Calculation of genomic equivalents (GE):

  $$\frac{78.32}{40} = 1.96\ GE/\mu l$$

- Calculation of concentration:

  $$1.96 \times 6\ pg = 11.76\ pg/\mu l$$

Calculation of concentration/reaction (1 reaction = 5 µl DNA sample):

- Calculation of copy numbers:

  $copies/\mu l \times 5 = 78.32\ copies/\mu l \times 5 = 391.6\ copies/reaction$

- Calculation of genomic equivalents (GE):

  $GE/\mu l \times 5 = 1.96\ GE/\mu l \times 5 = 9.8\ GE/reaction$

- Calculation of concentration:

  $pg/\mu l \times 5 = 11.76\ pg/\mu l \times 5 = 58.8\ pg/reaction$

PCR Equipment and detection channel setup

The **InviQuant GeneCount 40** is validated for the following real time PCR devices.

Real-time PCR device	Detection	Detection-Channel	Dark-Quencher	Comment
Roche LightCycler® 480 II	InviQuant GeneCount 40	465/510	+	
	IAC	533/580	+	
Roche cobas z 480 Analyzer	InviQuant GeneCount 40	465/510	+	
	IAC	540/580	+	
Roche LightCycler® 2.0	InviQuant GeneCount 40	530	none	SureCC Color Compensation Kit II (Art.-No. F4010) is required
	IAC	560	none	
Applied Biosystems 7500	InviQuant GeneCount 40	FAM	none	Check the passive reference option ROX is none
	IAC	VIC	none	
Agilent MX3005P	InviQuant GeneCount 40	FAM	+	
	IAC	HEX	+	
Bio-Rad CFX96	InviQuant GeneCount 40	FAM	+	
	IAC	HEX	+	
Qiagen Rotor-Gene Q	InviQuant GeneCount 40	Green	+	
	IAC	Yellow	+	
Cepheid SmartCycler®	InviQuant GeneCount 40	FAM	+	
	IAC	VIC	+	
LTF MyGo	InviQuant GeneCount 40	dye specific	+	For analysis dye-specific calibration files are necessary. If these are needed, they can be requested.
	IAC		+	

Troubleshooting

Issue	Probable cause	Comments and suggestions
Positive Control negative	Pipetting Inappropriate storage of kit components	Repeat the PCR run Check pipetting scheme and the set-up of the reaction Aliquot reagents Store the **InviQuant GeneCount 40** at -20 °C and keep the Reaction Mix protected from light. Avoid repeated freezing and thawing
No Template Control positive.	Cross contamination	Replace all critical reagents Repeat the experiment with new aliquots of all reagents Always handle samples, components and consumables in accordance with commonly accepted practices to prevent carry-over contamination
Fluorescence intensity too low	Very low initial amount of target DNA	Increase the amount of sample DNA Note: Depending on the chosen method of DNA preparation, inhibitory effects may occur Dilute the sample and run the assay with the diluted sample. If the inhibition decreases, then it is likely there are PCR inhibitors in the sample.
Inaccurate dilution series (R value < 0.99)	Inaccurate sample and reagent pipetting The standard curve may not have been properly analyzed	Check pipetting scheme and the set-up of the reaction. Reanalyze the standard curve

Appendix I

General sample storage

Samples should be store at conditions and temperature, which avoid the degradation of DNA. We recommend for short time (2-4 hours) storage at 2 - 8 °C. The recommendation for longer storage is at –80 °C or at least -20 °C. However, take care a repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.

Starting material for DNA extraction

Serum and plasma: After collection and centrifugation, serum, plasma, from blood (treated with anticoagulants like EDTA or citrate, but not with heparin), synovial fluid samples or other cell free body fluids, swabs as well as stool samples can be stored on ice for 1 - 2 hours, for short time (up to 24 h) samples may be stored at 2 - 8 °C. For long term storage, we recommend freezing samples in aliquots at –80 °C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing before DNA isolation should be avoided. In addition, cryoprecipitate formed during freeze-thawing could cause problems. If cryoprecipitate is visible, they should be pelleted by centrifugation at app. 6.800 x g for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately.

Paraffin slices / formalin-fixed tissue: These samples can be stored at room temperature (RT). By appropriate paraffin embedding or formalin fixation pure DNA can be isolated from above named starting material, but paraffin embedding or formalin fixation leads to reduced DNA quality. An improper contact of the tissue with formalin will reduce the yield of DNA dramatically.

Blood: Human blood samples (stabilized with EDTA or Citrate) can be stored at room temperature for 2 - 3 hours, for short time storage (up to 24 h) samples may be stored at 2 - 8° C. For long-term storage, we recommend freezing samples at – 20 °C or – 80 °C. Multiple thawing and freezing before isolating the DNA should be avoided. If cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the RTA Spin Filter membrane. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Blood Cards: Drying blood on filter paper is an effective form of storage. Samples prepared in this manner are cheaper and safer to transport. A sample (3 mm^2) punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 2 pieces of 3 mm^2 as starting material.

Swabs, Saliva: The protocol works with fresh saliva, prepared swabs as well as with dried swabs for isolation of total (genomic and mitochondrial) DNA. Please note, that stored and dried swab sample often characterized by isolation of apoptotic DNA (visible on agarose gel as typical apoptotic DNA ladder). The protocol has not been validated for isolation of DNA from swabs, which are stored under special storage buffers of other providers. Swabs may be processed on the same day as collected or stored for future processing, while storage at –20°C is recommended. DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months. These swabs or brushes are to air-dry for at least 2 h after sample collection.

Storage of forensic samples

Nail Scrapings: Forensic nail-scraping samples (old, fresh) are a source for total (genomic and mitochondrial) DNA isolation, can be stored at RT for long time. The amount of biological sample material should not exceed 10 mg.

Sperm: Best results are obtained with as fresh as possible material or material that has been as soon as possible frozen and stored at – 20 °C.

Chewing Gum: Chewing Gum (dried, old, fresh) is a source for total (genomic and mitochondrial) DNA isolation. The Chewing Gum can be stored under dry conditions at 4 °C for some weeks. For long-term storage, store them at –20 °C. The amount of sample material should not exceed 10 -20 mg.

Cigarette Butts: DNA from forensic cigarette-butt samples based on the isolation of total (genomic and mitochondrial) DNA from saliva and epithelial cells on paper from cigarette butts. Store the cigarette-butts under dry conditions at 4 °C for some weeks. For long term storage store them at –20 °C.

Postage Stamps and Envelopes: The isolation of DNA from stamps and envelopes is based on the purification of total (genomic & mitochondrial) DNA from saliva and epithelial cells on the paper of the stamps and envelopes. Store the stamps/ envelopes under dry conditions at 4 °C for some weeks. For long-term storage, store them at –20 °C.

Stains on Fabric: Stains (e.g., blood- or saliva) on fabrics or leather (~0.5 cm^2) should be stored under dry conditions at 4 °C for some weeks. Long-term storage is to be done at –20 °C.

Human Tissues/ frozen section: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20 °C or -80 °C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material influences yield of purified DNA. The amount of purified DNA using 5-10 mg tissue sample, depends on kind of starting material. The thawing process could proceed directly in the Lysis Buffer.

Hair: Best results are obtained from the hair roots of plucked hair samples (genomic and mitochondrial DNA) from hair without roots only mitochondrial DNA is available. Hairs should be stored under dry conditions at RT or for long-term storage store them at –20 °C.

Bones or Teeth: The best results are obtained from fresh bones or teeth (genomic and mitochondrial DNA). For long-term storage, they should be stored clean and under dry conditions at RT.

Appendix II

Kits for DNA extraction that can be used in combination with the **InviQuant GeneCount 40** assay:

Product	Cat. No	Package Size
Invisorb® Spin Blood Mini Kit for rapid purification of genomic DNA from 1 - 200 µl whole mammalian blood (with the common anticoagulants: EDTA, citrate) and for up to 25 µl of non mammalian blood; blood stains, swabs and bone marrow	1031100300	250 preps
Invisorb® Spin Tissue Mini Kit for purification of genomic DNA from 0.5 - 40 mg tissue sample; mouse tail or paraffin embedded/ formalin fixed tissue; 10 – 10^6 eucaryotic cells; swabs as well as from food samples of animal origin	1032100300	250 preps
Invisorb® Spin Forensic Kit for purification of genomic DNA from forensic samples, like small human whole blood samples,blood cards (dry blood stain), biopsy samples, body fluids, nails, hair roots, teeth, bones et al.	1034110300	250 preps
PSP® SalivaGene DNA Kit for purification of total DNA from SalivaGene® stabilized clinical swab (mouth brushes) and saliva sample	1035200300	250 preps
PSP® Spin Stool DNA Kit for purification of total DNA from fresh or frozen stool samples	1038100300	250 preps
PSP® Spin Stool DNA *Plus* Kit for collection, storage, stabilization and purification of total DNA from stool samples including Stool Collection Tubes with Stool DNA Stabilizer	1038110300	250 preps
Invisorb® Blood Mini HTS 96 Kit/ C for purification of genomic DNA from up to 200 µl fresh/ frozen human whole blood samples or from 75 µl mammalian blood samples etc. (with the common anticoagulants: EDTA, citrate) in a 96-well format using a centrifuge	7031300400	24 x 96 preps
InviMag® Blood DNA Mini Kit/ IG **(for use on the InviGenius® and InviGenius® PLUS, Invitek Molecular GmbH)** for fully automated purification of DNA from up to 200 µl whole blood samples (with the common anticoagulants: EDTA, citrate) with magnetic beads	2431120100	8 x 12 preps
InviMag® Blood DNA Mini Kit/ KF96 **(for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher Scientific)** for automated purification of total DNA from up to 200 µl of whole blood samples, buffy coat, non-mammalian blood (with the common anticoagulants: EDTA, citrate), bone marrow and swabs with magnetic beads	7431300200	5 x 96 preps
InviMag® Free Circulating DNA Kit/ IG **(for use on the InviGenius® PLUS, Invitek Molecular GmbH)** for fully automated purification of free circulating DNA from serum/ plasma with magnetic beads	2439320400	8 x 12 preps
InviMag® Stool DNA Kit/ KF96 **(for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher Scientific)** for automated purification of total genomic DNA from up to 200 mg fresh or frozen stool samples with magnetic beads	7438300200	5 x 96 preps

Ordering Information

Product	Package size	Catalogue No.
InviQuant GeneCount 40	200 reactions	3130100100

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