# InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit for Use with non-7500 Instruments – User Guide v1.2

For use with:

InnoQuant® HY-R (product numbers: 21104-250, 21104-60)

## Table of Contents

## InnoQuant® HY–R Kit Contents for 250 reactions (Item # 21104-250)

| | | |
|---|---|---|
| InnoQuant® HY-R Primer Mix | 2 red top tubes | 1 mL each |
| DNA Standard, 100 ng/µl | 1 clear top tube | 75 µL at 100 ng/µL |
| InnoQuant® Dilution Buffer A | 2 blue top tubes | 1.8 mL each |

Agilent Technologies Brilliant Multiplex QPCR Master Mix (Agilent Cat #600553):

| | | |
|---|---|---|
| Master Mix | 1 vial | 2.5 mL |
| ROX reference dye | 1 green top tube | 100 µL at 1 mM |

## InnoQuant® HY–R Kit Contents for 60 reactions (Item # 21104-60)

| | | |
|---|---|---|
| InnoQuant® HY-R Primer Mix | 1 red top tube | 0.5 mL each |
| DNA Standard, 100 ng/µl | 1 clear top tube | 33 µL at 100 ng/µL |
| InnoQuant® Dilution Buffer A | 1 blue top tube | 1.8 mL each |

Agilent Technologies Brilliant Multiplex QPCR Master Mix (Agilent Cat #5190-7274):

| | | |
|---|---|---|
| Master Mix | 1 vial | 0.5 mL |
| ROX Reference Dye | 1 green top tube | 100 µL at 1 mM |

NOTE: The **InnoQuant® HY-R Kit** has been validated and optimized for use with Agilent Technologies Brilliant Multiplex QPCR Master Mix.

## Storage Conditions

Upon receipt, store the kit at -20°C. After thawing, store all components at 2°C to 8°C.

**IMPORTANT**! The fluorescent dyes attached to the probes are light-sensitive. Protect the InnoQuant® HY-R primer mix and Agilent reference dye from light when not in use.

*Please note that the InnoQuant® HY-R Kit is designed to be highly sensitive. All reasonable precautions including clean laboratory techniques should be employed.*

## I. Preparation of DNA Standards

1. Equilibrate InnoQuant® HY-R DNA Standard (100 ng/µL) to room temperature for at least 15 minutes. Gently vortex for 5 seconds and centrifuge for 10–15 seconds at 3000 rpm before use.

**Caution**: The InnoQuant® HY-R Primer Mix contains probes that are labeled with light-sensitive dyes. Thaw completely at room temperature protected from light.

2. Using InnoQuant® HY-R Dilution Buffer A, prepare fresh serial dilutions of the InnoQuant® HY-R DNA Standard using low adhesion tubes as indicated below. Before removing an aliquot for the next dilution, gently vortex for 5-10 seconds and pulse spin for 5-10 seconds to collect all liquid at the bottom of the tube. The standard dilution series is a 5-point 1:8 dilution and ranges from 20 ng/µL (Std. 1) to 0.005 ng/µL (Std. 5).

**Note:** Once the standard is thawed, keep at 2-8 °C. Subjecting the InnoQuant® HY-R DNA Standard to multiple cycles of freeze-thaw is not recommended.

**Note:** Diluted DNA quantification standards can be stored in LOW ADHESION TUBES for up to 1 week at 2-8 °C. Longer term storage is not recommended.

**Note:** Be sure to change pipette tips between dilutions. Change gloves after handling high-concentration DNA.

| Standard | Concentration (ng/µL) | Recommended Dilution Amounts | Dilution Factor |
|---|---|---|---|
| Std. 1 | 20 | 10 µL [stock 100 ng/µL] + 40 µL Dilution Buffer A | 5X |
| Std. 2 | 2.5 | 10 µL [Std. 1] + 70 µLDilution Buffer A | 8X |
| Std. 3 | 0.3125 | 10 µL [Std. 2] + 70 µL Dilution Buffer A | 8X |
| Std. 4 | 0.0391 | 10 µL [Std. 3] + 70 µL Dilution Buffer A | 8X |
| Std. 5 | 0.005 | 10 µL [Std. 4] + 70 µL Dilution Buffer A | 8X |

## II. Sample Setup and PCR Conditions

1. Equilibrate InnoQuant® HY-R Primer Mix, InnoQuant® HY-R Dilution Buffer A, and Agilent Brilliant Multiplex QPCR Master Mix to room temperature for at least 15 minutes in the dark.

**Note:** Protect all tubes containing the Primer Mix from light as much as possible.

2. Gently agitate the Master Mix for 3-5 seconds to mix and briefly vortex the Primer Mix for 3-5 seconds. Gently tap to get all liquid to the bottom of tube.

**Note:** Do not centrifuge Primer Mix after vortexing, as this may cause the concentration of primers at the bottom of the tube.

3. Determine the number of reactions to be set up, including negative control reactions and 2 additional reactions to provide excess volume for the loss that occurs during reagent transfers.

4. Prepare the reaction mix by combining the Master Mix and Primer Mix as indicated below and vortex briefly.

| PCR Components | Volume per Reaction |
|---|---|
| Agilent Brilliant Multiplex QPCR Master Mix | 10 µL |
| InnoQuant® HY-R Primer Mix | 8 µL |
| **Total Volume** | **18.0 µL** |

5. In an optical plate, add 2 µL of DNA Standard or unknown sample to the appropriate wells. Make sure to place the sample at the bottom of the well. Duplicate amplification of standards is required. Add 2 µL of InnoQuant® Dilution Buffer A to the NTC reaction(s).

6. Add 18 µL of the reaction mix to each appropriate well of an optical-grade PCR plate. Mix the reaction by pipetting up and down 4-5 times as the reaction mix is being added to each well.

7. Seal the plates with an optical adhesive cover using the plate cover applicator.

8. Centrifuge the plate for 1 minute at 1500 rpm to collect the contents of the wells at the bottom. If bubbles are still present after centrifuging, repeat this step one time.

**Note:** Protect the plate from extended light exposure or elevated temperatures before cycling. Handle the plate by the edges, and avoid touching the adhesive cover and bottom of the plate.

9. Verify the Cycling Parameters:

| | |
|---|---|
| Initial heating time: | 10 min at 95 °C |
| Followed by 40 cycles of: | 15 seconds at 95 °C |
| | 2 minutes at 61 °C |

## III. Result Interpretation

| | | |
|---|---|---|
| **Short Target** | HEX: | 80 bp size |
| **Long Target** | Cy5: | 207 bp size |
| **Y Target** | FAM: | 80 bp size |
| **Internal Positive Control (IPC)** | ROX: | 172 bp size |

**Run Parameters**

The following parameters should be evaluated for each run:

1. Passing reaction efficiency values are greater than 90% and less than 110% for the Short, Long, and Y targets. Acceptable slope values are between -3.6 and -3.1.

   The $C_T$ threshold and baseline parameters may be edited if the slope or $R^2$ values are outside the acceptable ranges. Ensure the threshold is within the exponential phase of the amplification plot.

2. Passing $R^2$ values are greater than 0.98.

3. The IPC is expected to have $C_T$ values no more than 2 units above the mean IPC $C_T$ for all quantification standards on the plate. $C_T$ values higher than these for the IPC is indicative of a PCR inhibitor present in the reaction, including an excessively high concentration of DNA.

**NOTE** regarding NTCs and negative controls: The InnoQuant® HY-R assays are highly sensitive. Spurious signals may be obtained which are possibly the result of ambient DNA or sporadic signal from the short or long targets. Sporadic signal is more likely to be observed with the short and the long targets than the Y target due to the higher copy number. For this reason, it is recommended to disregard signals in NTCs or negative controls higher than 30 $C_T$ for the short and long InnoQuant® HY-R targets.

IMPORTANT! Before using the highly sensitive InnoQuant® HY-R Kit, assess the cleanliness of your environment. Use stringent laboratory cleanliness protocols to minimize contamination.

**Sample Analysis**

To obtain the Degradation Index, divide the short quantity value by the long quantity value:

$$DI = \frac{[Short]}{[Long]}$$

## IV. Troubleshooting

| Observation | Possible Causes | Recommended Actions |
|---|---|---|
| IPC $C_T$ falls outside acceptable range | High concentration of EDTA | Verify the EDTA concentration in the unknown samples is 0.1mM EDTA. |
| | PCR inhibition | Purify DNA extracts using Microcon filtration units and repeat the quantitation. Alternatively, quantify a dilution of the sample DNA extract (i.e. 1/10 or 1/100 dilution). |
| | Analysis parameters incorrect | Ensure the baseline and threshold analysis parameters are set correctly in the software (under the Analysis Settings tab). |
| Low Efficiency values or slope values outside the acceptable range | Primers/probes not properly thawed | Allow primers/probes to equilibrate to room temperature for 15 minutes |
| | DNA standards too old | Re-dilute standards from100ng/µl stock using InnoQuant® Dilution Buffer A. |
| Low $R^2$ values | Incorrect threshold setting | Verify the threshold set is in the exponential phase of the amplification curve and above baseline |
| | Pipette maintenance (or possible pipetting error) | Check maintenance of pipettes for calibration |
| S-shaped target standard curve | Incorrect threshold setting | Verify the threshold set is in the exponential phase of the amplification curve, and that the baseline settings are adequate, and reanalyze |

*NOTE: During the development of products for forensic DNA analysis, InnoGenomics Technologies performs developmental validation studies. However, it is the responsibility of the customer laboratory to perform its own analysis and internal validation studies, and develop its own standard operating procedures and interpretation guidelines, to ensure that the products and services it obtains from InnoGenomics Technologies satisfy or will satisfy the applicable guidelines used by the forensic community and are fit for the customer laboratory's human identification applications.*



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