HERA^{PLUS} SYBR® Green qPCR Kit

(WF1030800X)

Overview

The *HERA*^{PLUS} SYBR® Green qPCR kit allows genotyping of dsDNA samples using SYBR® dye. The one-step approach to real-time PCR allows for the full reaction to occur in one tube, limiting the potential for contamination and improving reproducibility through reduced sample handling. The *HERA*^{PLUS} SYBR® Green qPCR provides a convenient and powerful method for DNA detection and quantitation, allowing for gene expression analysis and population genotyping. In addition to the highly sensitive SYBR® Green dye, *HERA* is formulated with a unique ROX passive reference dye that is compatible across a variety of instrument platforms. However, unlike our other *HERA* products, the user will not have to select the intensity of the reference dye, improving the setup time.

Stages

1. *HERA*^{PLUS} Enzyme Activation
2. Cycle denaturation
3. Primer annealing and extension

Features

Efficient – Using SYBR® Green in the master mix allows the detection of the amplicon with high sensitivity and guarantees the linearity of the qPCR

High yield – The ready-to-use master mix ensures optimal performance of *HERA* Taq Polymerase to maximise DNA yield.

Robust – High fidelity amplification with little optimisation required.

Product information

Catalog number	Package Size
WF10308001	100 reactions
WF10308002	500 reactions
WF10308003	1000 reactions

Required materials

- dsDNA template
- Specific gene Forward and Reverse primers
- Pipettes and tips
- Vortex
- 0.2mL PCR tubes
- Ice
- Nuclease Free Water
- qPCR Thermocycler
- Centrifuge

Reaction timings

Once each reaction tube has been set up, the *HERA*^{PLUS} SYBR® Green qPCR kit will be complete in a minimum of 29 minutes. This all-in-one protocol allows for a time-sensitive reaction and quick genetic data analysis.

Storage conditions

The reagents provided in the *HERA*^{PLUS} SYBR® Green qPCR Kit should be stored at -20^{0}C. Where possible stored reagents in conditions of non-frost-free.

Warning!

Please be advised to wear appropriate eyewear, clothing and gloves. Read the Safety Data Sheets (SDS) for any further safety information.

Any further information

For further information and protocols, visit our website https://willowfort.co.uk/. For any additional support, please contact us through https://willowfort.co.uk/contact-us

For research use only. These products should not be used for diagnostics.

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Components of *HERA*^{PLUS} SYBR® Green qPCR Kit

HERA^{PLUS} SYBR® Green qPCR Master Mix

HERA^{PLUS} **Hot Start Taq DNA Polymerase** – a thermostable DNA polymerase used for DNA synthesis with hot start activity.

HERA^{PLUS} **SYBR® Green qPCR buffer –** contains the optimal concentrations of $(NH_4)_2SO_4$ and KCl and is buffered to pH9 to maximise performance. Ready-to-use master mix compatible with both fast and standard cycling methods on most thermal cyclers. It is designed to normalise with no, low and high ROX instruments.

- **Antibodies** – Included in the *HERA* buffer. To prevent amplification of non-specific products we have used antibodies which are designed to inactive the enzyme during primer annealing phases of the reaction.
- **$MgCl_2$** – This is included within the *HERA* buffer since the polymerase requires Mg^{2+} as a cofactor to function. If the DNA template has been extracted with chelating agents such as EDTA, additional Mg^{2+} ions are required to maintain enzyme performance. We recommend adding 5µl of 25mM $MgCl_2$ *(not included).*
- **dNTPs** – This is included in the *HERA buffer.* The purity of dNTPs in all Willowfort products is ≥99%, to ensure maximum fidelity and speed. For longer length amplicons, additional dNTPs maybe required *(not included, but can purchased on our website).*
- **Storage buffer –** Included in the *HERA* buffer. 20mM Tris buffer pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT and glycerol to prevent enzyme degradation.

Protocol for *HERA* SYBR® Green qPCR kit

1. Thaw all reagents and store on ice. Mix and centrifuge all reagents well before preparing the master mix. Check for precipitation
2. Combine all reagents together in a nuclease free eppendorf, using the table 1 as a guide. Mix thoroughly and briefly centrifuge.

Table 1: Reagents required per sample

Component	Amount
HERA^{PLUS} SYBR® Green Master Mix (2x)	10µl
Forward primers 20x (200nM)	1µl (up to 1µM each)
Reverse primers 20x (200nM)	1µl (up to 1µM each)
Nuclease free water	To 20µl
DNA template (up to 250ng)	Volume varies

3. Aliquot the master mix into separate 0.2mL PCR tubes before adding the DNA template.
4. Gently mix together and centrifuge briefly.
5. Use Table 2 as a guide to set up the thermal profile, considering the melting temperature of the primers. Set ROX as the reference dye.

Table 2: Temperature and Times for the PCR protocol

Stage	Temperature	Time
Stage 1	95˚C	2 min
Stage 2.1	95˚C	*10sec
Stage 2.2	60˚C	20-30sec
Repeat stage 2 for 40 cycles		

*Increase to 1min, if GC content is higher than ≤50%

6. Add and incubate reactions into the qPCR thermocycler. During the last stage, *HERA* SYBR® Green intercalates with the amplicon to generate a fluorescent signal, which is detected by the qPCR instrument.
7. Once the reaction is complete, Ct value data can be analysed for gene expression.

Additional protocol information

Tm calculation – For primers below 25 nucleotides, please use this equation to calculate the Tm:

$$Tm = 4(G+C) + 2(A+T)$$

If the primer has more than 25 nucleotides this equation is insufficient, and computational methods are required.

Template – Use up to 250ng of template DNA to minimise errors in the product.

Troubleshooting – For any troubleshooting queries, please visit our website http://willowfort.co.uk/quantitative-pcr/hera-plus-sybr-green-qpcr-kit. Any further information, please contact us, via https://willowfort.co.uk/contact-us.