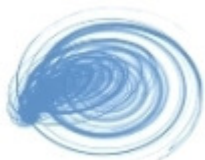


PrimerDesign Ltd

***Precision*TM HRM
Mastermix
&
*Precision*TM raZor Mix**

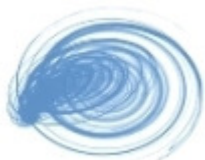
Mastermixes for **High Resolution Melting** analysis





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Introduction

PrimerDesign Ltd *Precision HRM™* Mastermixes are optimized for use in real-time PCR followed by high resolution melting analysis. The Mastermix contains a thermo-stable TAQ Polymerase as well as a buffer and $MgCl_2$ at concentrations optimised for the enzyme. In addition, the Mastermix contains dNTPs required for amplification of a DNA target.

This complete solution requires only the addition of your gDNA, and genotyping primers to be PCR ready.

Precision HRM™ Mastermixes are unique in containing our high spec HRM specific dye. This dye has saturating binding properties and is very stable. Together these attributes make *Precision HRM™* Mastermix the ideal choice for sensitive post PCR melting analysis.

Precision HRM™ Mastermix is suitable for all HRM applications whilst *Precision raZor* mix is recommended for use alongside our HRM raZor probe assays.

Guide to Hardware compatibility

Manufacturers use varying methods to calibrate a real-time PCR reaction. For this reason the correct *Precision HRM™* Mastermix formulation must be used for each platform.

| Cat Number | Product Description | Compatible Hardware |
|---------------------|--|---|
| Precision-HRM | <i>Precision HRM™</i> MasterMix | Rotorgene 6000 |
| Precisionblue-HRM | <i>Precision HRM™</i> MasterMix with blue dye | Roche lightcycler 480 Platform and others |
| Precision-razor | <i>Precision raZor</i> Mix for use with raZor probe assays | Rotorgene 6000 |
| Precisionblue-razor | <i>Precision raZor</i> Mix for use with raZor probe assays with blue dye | Roche lightcycler 480 Platform and others |



Kit Contents

- 4 x 1.6ml aliquots of *Precision HRM*[™]
or
- 4 x 1.6ml aliquots of *Precision HRM*[™] raZor mix

Recommended Accompanying Products

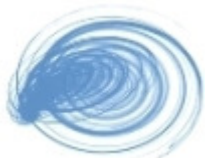
- PrimerDesign custom designed HRM analysis assay
- PrimerDesign custom designed HRM raZor probe assay

Reagents and Equipment to Be Supplied by User

- Real-Time PCR Instrument with HRM functionality
- Pipettors and Tips
- Vortex and centrifuge

Kit Storage

The PrimerDesign *Precision HRM*[™] Mastermix kit should be stored at -20°C on arrival. Repeated freeze/thawing will not compromise the performance of the product. Under these conditions reagents are stable for six months from date of receipt.



Suitable Sample Material

Please ensure that gDNA samples are suitable in terms of purity, concentration and DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the test sample gDNA with RNase/DNase free water.

Licensing Agreement and Limitations of Use

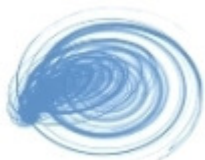
PCR is covered by several patents owned by Hoffman-Roche Inc and Hoffman-LaRoche, Ltd. Purchase of PrimerDesign kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others. SYBR[®] green is a registered trademark of Molecular Probes Inc.

PrimerDesign Ltd Satisfaction Guarantee

PrimerDesign takes pride in the quality of all our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, PrimerDesign will replace the item free of charge.

Quality Control

As part of our routine quality assurance programme, all PrimerDesign products are monitored to ensure the highest levels of performance and reliability.



Bench-side Protocol

When using PrimerDesign genotyping primer kits.

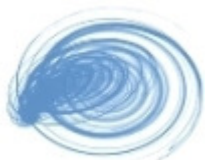
For each 15µl real-time PCR reaction add the following to each reaction tube

| Components | 1 Reaction |
|--|--------------|
| <i>Precision HRM</i> TM Mastermix | 7.5 µl |
| Primer mix | 1 µl |
| Template (2-10ng genomic DNA) | x µl |
| RNAse/DNAse free water | x µl |
| Final volume | 15 µl |

Suggested use with user supplied primers.

For each 15µl real-time PCR reaction add the following to each reaction tube

| Components | 1 Reaction |
|--|--------------|
| <i>Precision HRM</i> TM Mastermix | 7.5 µl |
| Primers | x µl |
| Template (2-10ng genomic DNA) | x µl |
| RNAse/DNAse free water (up to Final volume) | x µl |
| Final volume | 15 µl |



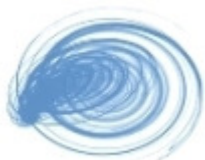
Amplification Protocol

Amplification conditions using PrimerDesign primer only HRM assays.

| | Step | Time | Temp |
|--------------|-------------------------|-------------------------------|-------------|
| | Enzyme activation | 8min | 95°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 66°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 64°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 62°C |
| Cycling x 30 | Denaturation | 15s | 95°C |
| | DATA COLLECTION* | 45s | 60°C |
| HRM | Denaturation | 45s | 95°C |
| | Rapid cooling | rapid | 55 °C |
| | HRM Melt Curve** | According to machine protocol | |

*Fluorogenic data should be collected during this step through the green (SYBR® green/FAM) channel.

** perform HRM analysis + and – 5 °C across the amplicon TM (this information in on the custom datasheet supplied with the assay).

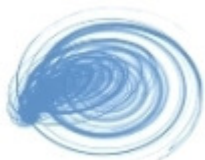


Amplification conditions using PrimerDesign gene scanning HRM assays.

| | Step | Time | Temp |
|---------------------|-------------------------|-------|-------------|
| | Enzyme activation | 8min | 95°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal | 20s | 66°C |
| | extend | 20s | 72°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal | 20s | 64°C |
| | extend | 20s | 72°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal | 20s | 62°C |
| | extend | 20s | 72°C |
| Cycling x 30 | Denaturation | 15s | 95°C |
| | Anneal | 20s | 56°C |
| | DATA COLLECTION* | 20s | 72°C |
| HRM | Denaturation | 60s | 95°C |
| | Rapid cooling | rapid | 55 °C |
| | HRM Melt Curve | ** | ** |

*Fluorogenic data should be collected during this step through the SYBR® green channel.

** perform HRM + and – 5 °C across the amplicon melting point (the amplicon T_m is included on the custom datasheet for each primer set)



Amplification conditions using PrimerDesign raZor[®] probe HRM assays.

| | Step | Time | Temp |
|---------------------|-------------------------|-------------|-------------|
| | Enzyme activation | 8min | 95°C |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 66°C |
| | | | |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 64°C |
| | | | |
| Cycling x 3 | Denaturation | 15s | 95°C |
| | Anneal and extend | 45s | 62°C |
| | | | |
| Cycling x 50 | Denaturation | 15s | 95°C |
| | DATA COLLECTION* | 45s | 60°C |
| | | | |
| HRM | Denaturation | 45s | 95°C |
| | Rapid cooling | Max ramp | 55 °C |
| | HRM Melting Curve** | | |

*Fluorogenic data should be collected during this step through the green (SYBR[®] green/FAM) channel.

** perform HRM analysis + and – 5 °C across the amplicon T_M (this information is on the custom datasheet supplied with the assay).