Kit No. HRLS-ASY-0002 100 Reactions
Kit No. HRLS-ASY-0003 500 Reactions

LightScanner®
Master Mix
User's Guide

BIOFIRE DEFENSE

Designed for Hi-Res Melting

Kit Contents

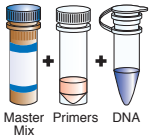
Quantity	Description
1 x 100 Reactions OR 5 x 100 Reactions	2.5X Master Mix
1.5 mL	10 mM $MgCl_2$
1.5 mL	Reagent Grade Water
1	LightScanner Master Mix User's Guide

LightScanner® Master Mix LSCN-PRT-0023-07 01/19

Protocol at a Glance

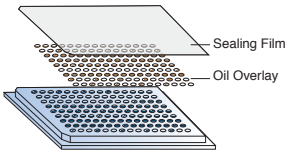
Step 1: Wet Reaction Setup

Follow basic PCR reaction setup using the Master Mix, primers and DNA. The Master Mix contains LCGreen® Plus.



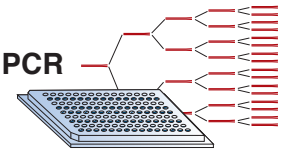
Step 2: Place Mix in Plate with Oil Overlay

Preload PCR plate with mineral oil, add wet mix, seal with film and centrifuge briefly at 2500 rpm.



Step 3: Thermocycle Samples

Follow the recommended protocols to perform PCR.

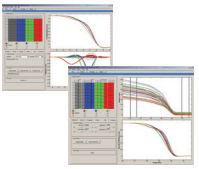


Step 4: Melt Samples in LightScanner

Following PCR, insert the 96- or 384-well plate into a LightScanner to melt the samples.

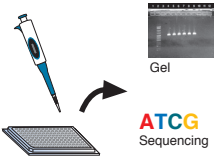
Step 5: Analyze Melt Data

Following the melt, use the LightScanner software to manage and analyze the data.



Step 6: Scanning is Nondestructive

Samples can be recovered for additional analysis, sequencing, gel electrophoresis, remelting, etc.



Approximately 5 to 8 Minutes

Overview of Workflow
About the LightScanner® Master Mix

This LightScanner Master Mix from BioFire Defense (Idaho Technology) provides a pre-optimized Master Mix specially designed for Hi-Res Melting® mutation discovery and mutation scanning on the LightScanner instrument.

This Master Mix incorporates a Taq polymerase and an antibody-based hot-start method, dNTPs, magnesium chloride, and BioFire's proprietary LCGreen® Plus dye and is formulated at a 2.5X concentration. The antibody hot-start method means that no enzyme preactivation is required before thermocycling.

The Master Mix includes a concentration of magnesium that is optimized to work for almost all reactions. Additional magnesium chloride solution is provided for further optimization if required. The Master Mix is also compatible with most additives used to enhance amplification efficiency of high GC amplicons.

Storage/Stability

The LightScanner Master Mix is stable for at least 1 year when stored at –20ºC. Once thawed it can be stored for up to 2 weeks at 4ºC with no loss of activity.

Equipment needed but not provided:
White-well PCR plates*
Sealing film*
Pipettes (1–10 µL , 200 µL)
Plate centrifuge
Thermocycler
DMSO (optional)

*See **Appendix A** for recommended PCR plates and sealing film

Reaction Setup

Thaw the frozen Master Mix solution on ice. Mix thoroughly before using. Once thawed, the Master Mix can be stored at 4°C for up to 2 weeks.

Recommended final reaction volumes:

- 4 µL Master Mix per reaction in a 10 µL final volume

Master Mix Volumes (10 µL final volume)

No. Reactions Required	Master Mix (µL)
1	4
10	40
48	192
96	384

Plate Setup

Preparation of PCR mix for **one** 10 µL reaction (8 µL template-free PCR mix + 2 µL DNA). These volumes can be scaled up as desired.

Component	Vol. (µL)	Final Concentration	Example for 10 Reactions (µL)
2.5X Master Mix	4	1X	4 x 10 = 40
10X Forward Primer	1	1X	1 x 10 = 10
10X Reverse Primer	1	1X	1 x 10 = 10
Water	2	N/A	2 x 10 = 20
Final Volume	**8**		80

- Mix the reagent gently but thoroughly before dispensing (e.g., pipette up and down and spin).

Note: *To prevent cross-contamination, 8 µL of the template-free PCR mix should be added to each well of the plate, then 2 µL of template DNA should be added to bring the reaction volume up to 10 µL.*

Load the plate as follows:

1. Aliquot **25 µL** of mineral oil into each well.
2. Aliquot template-free PCR mix into each well (8 µL per well).
3. Add DNA template to each well (2 µL per well).
4. Cover plate with sealing film.
5. Centrifuge 1-2 min. at 2000–3000 rpm.

Typical PCR Protocol

This amplification protocol can work for many PCR products in the size range between 150–400 base pairs.

Block Thermocycling Conditions			
	Temp. (°C)	**Time (sec.)**	**Step**
1 Cycle (optional)	95	120	Initial Template Denaturation
40–45 Cycles	94	30	Denaturation
	Primer Dependent	30	Annealing
Heteroduplex Formation			
1 Cycle	94	30	
	25–28	30	

Typical LunaProbes® Run Protocol

This amplification protocol can work for many PCR products in the size range between 150–400 base pairs.

Block Thermocycling Conditions			
	Temp. (°C)	**Time (sec.)**	**Step**
1 Cycle (optional)	95	120	Initial Template Denaturation
55 Cycles	94	30	Denaturation
	Primer Dependent	30	Annealing
	72	30	Extension*

*3-step PCR is necessary for genotyping applications

Typical LightScanner Run Protocol

This scanning protocol can work for many PCR products in the size range between 150–400 base pairs.

LightScanner Melting Settings	
Start Temperature	75°C*
End Temperature	94°C
Hold Temperature	3°C lower than start temperature
Exposure	Auto

*For genotyping applications decrease start temperature to 50°C.

APPENDIX A: Troubleshooting Guide for Chemistry

Symptom: Low overall fluorescence (the sample is weakly fluorescent).	
Probable Causes	**Solutions**
Plate map not aligned to wells of plate.	Use software alignment tool to align the map to the plate.
Acquisition of data was started after melting transition of fragment had already begun.	Adjust melting window to accommodate the melting characteristics of fragment.
PCR is not robust.	Assess robustness of PCR by gel electrophoresis. Adjust reaction chemistry ($MgCl_2$. Primer concentration, etc) or cycling parameters (annealing temperature, cycle number, etc) to increase robustness of reaction.
No oil overlay of single or scattered sample(s).	Set up PCR for those particular sample (along with needed controls) again.
No oil overlay for entire plate.	Make sure the entire plate is equally dehydrated (e.g. 90 ºC for 10 minutes on an uncovered thermocycler). Re-hydrate samples by adding water and oil overlay. Re-melt the samples.
Non-optically clear sealing film on the plate.	Replace with an optically clear film or remove altogether. See Appendix B for a list of optically clear plate films.

Symptom: Inconsistency of profiles between replicate samples or samples of common genotypes.	
Probable Causes	**Solutions**
Different DNA isolation chemistries (final elution buffer, storage buffer) used to prepare individual specimens.	Isolate DNA samples using a common protocol or bind existing DNA to silica and elute specimens into a common buffer.
Components of reaction mixture not consistent between samples (e.g., $MgCl_2$ concentration, primer concentration, adjuvant etc.).	Re-run with a consistent reaction composition among all of the samples.
Presence of nonspecific amplification products in the reaction.	Assess reaction by gel electrophoresis. Optimize the PCR chemistry and cycling conditions as described in Chapter 3.

Inconsistency of reaction volume between samples.	Re-run with a consistent reaction volume.
Inconsistent template DNA concentration between reactions.	Adjust template DNA specimens to be at a common concentration and add equal amount to each reaction.
Bubble present in a reaction well.	Centrifuge plate to remove bubble.

Symptom: Unexpected/Inconsistent low temperature melting inflections.	
Probable Causes	**Solutions**
Presence of nonspecific amplification product.	Assess reaction products by gel electrophoresis. Optimize the PCR chemistry and cycling conditions as described in the LightScanner System manual. Use a Hot-Start method.
Primer dimer product.	Evaluate primer design and re-design to eliminate dimer formation. Increase annealing temperature to make dimer formation less favorable. Reduce duration of annealing step to make dimer formation less favorable. Use a Hot-Start method.

Symptom: Unexpected/Inconsistent high temperature melting inflection (toeing).	
Probable Causes	**Solutions**
Presence of non-specific product having a higher melting temperature than the desired product.	Assess reaction by gel electrophoresis. If using a previously optimized PCR reaction, check for degradation of primer(s) by re-diluting from a stock and re-assess the reaction. Synthesize new primers if necessary. If using a non-optimized PCR reaction, optimize the PCR chemistry and cycling conditions as described in the LightScanner System manual.

Symptom: Difficulty analyzing high Tm PCR products.	
Probable Causes	**Solutions**
For PCR products that melt at high temperatures, it may be difficult to completely melt the products and get adequate high-temperature background for analysis.	In this case, consider adding DMSO (5-15%), either before (preferred) or after the PCR.

Notes

Notes

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