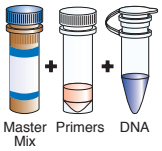
LightScanner® Master Mix Quick Guide

For Kits: HRLS-ASY-0002 100 Reactions | HRLS-ASY-0003 500 Reactions

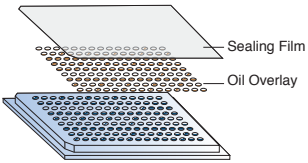
Step 1: Wet Reaction Setup

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



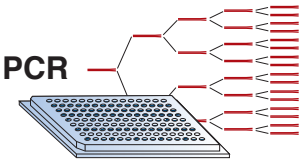
Step 2: Place Mix in Plate with Oil Overlay

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.

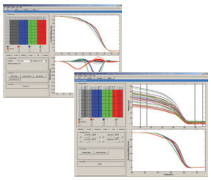


Step 4: Melt Samples in LightScanner

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

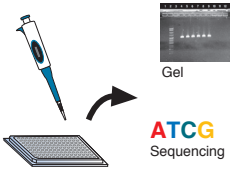
Step 5: Analyze Melt Data

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

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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. Add DNA template to each well (2 µL per well).
5. Centrifuge 1–2 min. at 2000–3000 rpm.