

# MELA Test Panel (100) reactions

*Research use only and not intended for human or animal, diagnostic or therapeutic uses.*

## Storage (-20° C)

### Kit (MELA – 2120 Braf V600E) ) Contains:

- MELA-MPCR Primers
- MELA-Sequencing Primers
- Enhancer
- Dnase free water

### Suggested Accessories:

- Big Dye Terminator (Life Technologies Version 1.1 Cat no. 4337450)
- Qiagen Multiplex master mix Cat No 206152
- Ampure-Xp (Beckman Coulter USA A 63881)
- CleanSeq (Beckman Coulter USA Cat No. 000121)

**Usage:** MultiGEN's MELA test panel detects Braf V600E mutation and wild type allele.

### Protocol:

MPCR Reaction	
REAGENT	Vol/Rx
Qiagen Buffer	25 ul
MPCR primers	2 ul
DNA Extract	10ul
Dnase free water	13 ul
Rx Vol	50 ul

Thermocycling profile	
Pre cycle 95° C	5 min
95° C	30 Sec
55.0° C	90 Sec
72° C	30 Sec
No. Cycles	35
Hold time	68° C/10 min

#### A. MPCR Clean up:

- Add 5 ul of the enhancer; incubate at 37° C for 60 min, heat at 95° C for 3 min, 4° C/hold.
- Purify MPCR reactions using Ampure according to the instructions provided (Beckman Coulter, USA).

Sequencing Reaction	
REAGENT	Vol/Rx
Big Dye	1.0ul
5X Sequencing Buffer	3.5 ul
Sequencing Primers	2.5 ul
Template	10.0 ul
Dnase free water	3.0 ul
Rx Vol	20 ul

Thermocycling profile	
96 <sup>0</sup> C	15 Sec
58.5 <sup>0</sup> C	15 Sec
60 <sup>0</sup> C	2.5 Min
No. Cycles	25
Hold temp	4 <sup>0</sup> C

- A. Cycle Sequencing Excess Dye Terminator Removal:** Purify cycle sequencing reactions using Cleanseq according to the instructions provided (Beckman Coulter, USA).
- B. Analysis:** Sequence the sample using capillary electrophoresis. Follow the manufacturer's instructions for the preparation of the sample. Using Sequencing Analysis Software Version 5.2 (Life Technologies, USA) and an ABI Prism™ 3130 Genetic Analyzer (Life Technologies, USA), the expected electropherogram is:

