Protocol

1. Set up two reactions for each testing sample

   Reaction#1: Total BRAF Assay for Testing Sample

Item	Volume
Total BRAF Assay Mix (10 x)	2μl
Reaction Master Mix (2 x)	20μl
DNA template	10-50 ng
PCR water	Add to 20μl
Total	20μl

   Reaction#2: BRAF Mutant Assay for Testing Sample

Item	Volume
BRAF Mutant Assay Mix (10 x)	2μl
Reaction Master Mix (2 x)	20μl
DNA template	10-50 ng
PCR water	Add to 20μl
Total	20μl

2. Set up two reaction mixes for control reaction (control assay)
   Control Assay#1: Total BRAF Assay for control template

Item	Volume
Total BRAF Assay Mix (10 x)	2μl
Reaction Master Mix (2 x)	10μl
Control template	2μl
PCR water	6μl
Total	20μl

   Control Assay#2: BRAF Mutant Assay for control template

Item	Volume
BRAF Mutant Assay Mix (10 x)	2μl
Reaction Master Mix (2 x)	10μl
Control template	2μl
PCR water	6μl
Total	20μl

3. Real-time PCR program

   3.1 Set the real-time PCR detector for FAM fluorescent dye and BHQ1 quencher.

   3.2 Run PCR as follows:

   a. 95˚C for 10 min - 1 cycle
   b. 95˚C for 15s, followed by 62˚C for 60s
      Total 40-45 cycles

4. Data analysis and validation

    1) Control Assay#1 should have a Ct value between 15-40.
    2) Control Assay#2 should have a Ct value ≥40.
    3) Reaction#1 (see above) for testing sample should have a Ct value between 15-40.
    4) If Reaction#2 for the testing sample has a Ct value ≤ 40, it is considered as positive for BRAF-V600E mutation.