Technical Sheet – LightMix® Digital EGFR C797S assay

Summary Information

Assay kit information

Product Cat.-No:	20-3007-32
Assay type:	Detection Kit for EGFR C797S mutations
Coverage:	EGFR C797S mutations c.2389T>A and c.2390G>C
Probe fluorophores:	FAM/HEX
Probe quenchers:	BHQ2
Primers/probes supplied as:	Air-dried oligo mix
Amplicon length:	113 bp
MIQE context sequence:	CGTGGACAACCCCCACGTGTGCCGCCTGCTGGGCATCTGCCTCACCTCCACCGT GCAGCTCATCACGCAGCTCATGCCCTTCGGC[T/A][G/C]CCTCCTGGACTATGTC CGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAACTGGTGTGTGCA GATCGCAAAGGTA
Positive control:	4-6% plasmid with COSM6493937 sequence insert in genomic DNA (K562 cell line) background.

Gene information

Gene name:	Epidermal Growth Factor Receptor
Gene symbol:	EGFR
Species:	Human
COSMIC ID for mutation:	COSM6493937 and COSM5945664

Verification information

Instrument:	Digital LightCycler
MasterMix:	Digital LightCycler 5x DNA Master
Restriction enzyme:	MseI, HindIII
Wild type template:	Human genomic DNA from blood (buffy coat)
Sequence variant template:	Plasmid (with COSM6493937 sequence insert)
Annealing temperature:	58˚C

Cycling protocol:

Step	Temperature (°C)	Time (sec)	Cycles
UNG activation	50	120	1
Denaturation	95	120	1
Amplification: *denaturation*	95	10	40
Amplification: *annealing/extension*	58	20	
Cooling	40	30	1

Stability

The stability of the reconstituted oligo mix has been tested for up to 60 days (stored in +2-8°C) and showed < 20% variability for 5% mutant samples (5% mutant in a background of 1cpp wild-type genomic DNA) in detected mutant concentration.

Template Input

Template input was varied between 0.5 and 2 copies per partition (cpp) to validate assay performance for different template loadings.

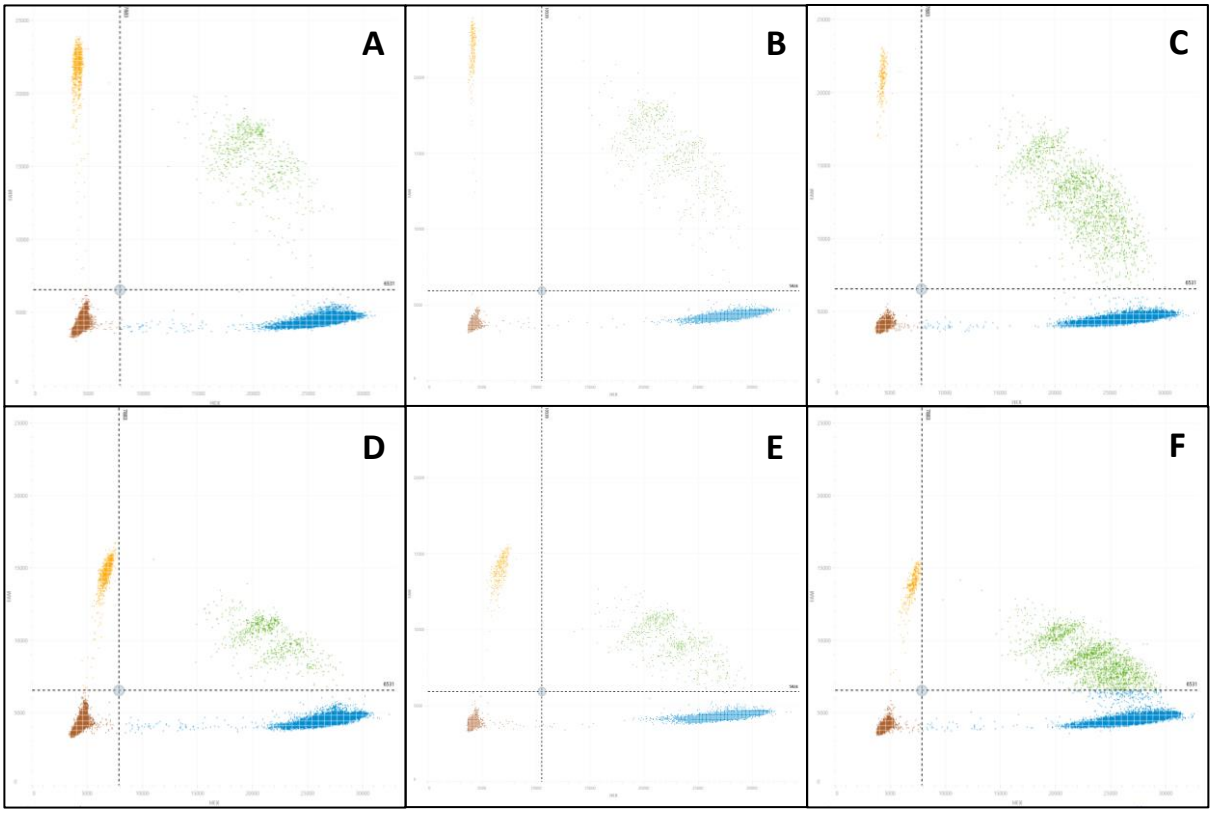

Figure 1. *2D scatter plot of samples with 5% G>C (**A**, **B**, **C**) or T>A (**D**, **E**, **F**) mutant template in genomic WT background with a total loading of: **A, D)** 0.5cpp, **B, E)** 1cpp and **C, F)** 2cpp. Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive*

Sensitivity

Varying amounts of synthetic mutant DNA were spiked into a 1cpp background of wild-type genomic DNA. The contrived samples ranged from 0.05% to 5% mutant spike-in. Blank samples and samples with only genomic DNA (0% mutant) were also included as negative controls. Analytical sensitivity was estimated to be <0.1% mutant based on a limit of blank (LOB) of 0.005% and the lower end of the CI95% for the 0.05% mutant sample above this LOB.

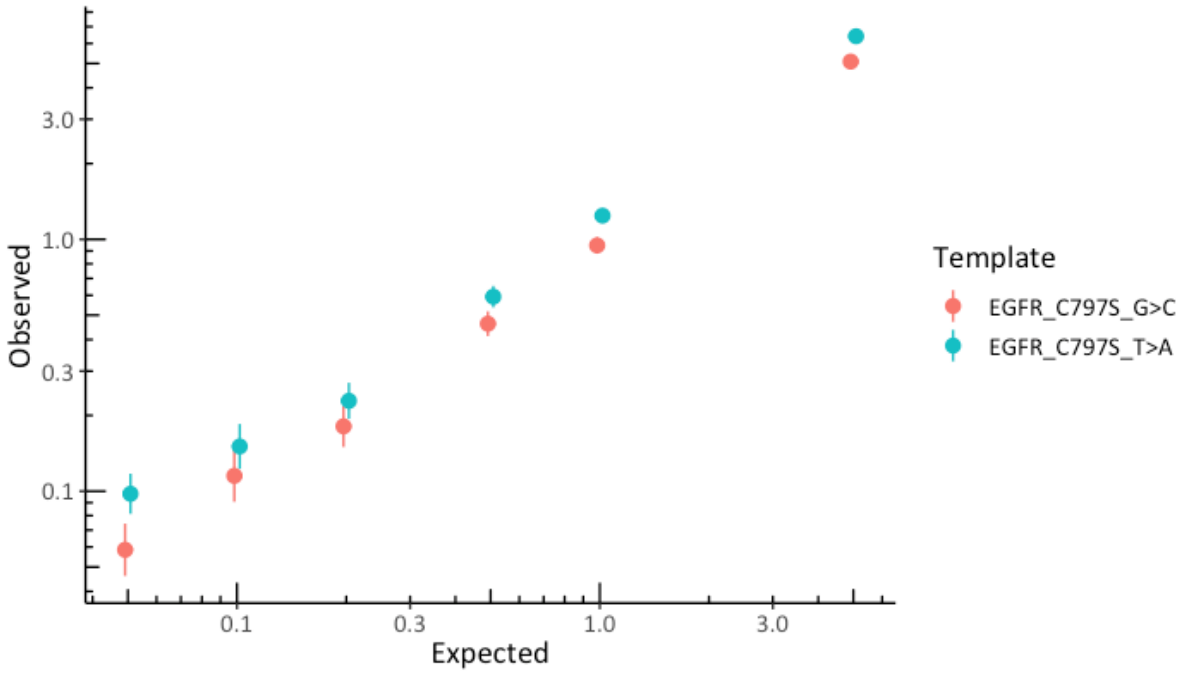

Figure 2. *Percentage mutant detected in the sample with 0.05% to 5% mutant in a WT genomic background (error bars = CI95%, axis in log-scale)*

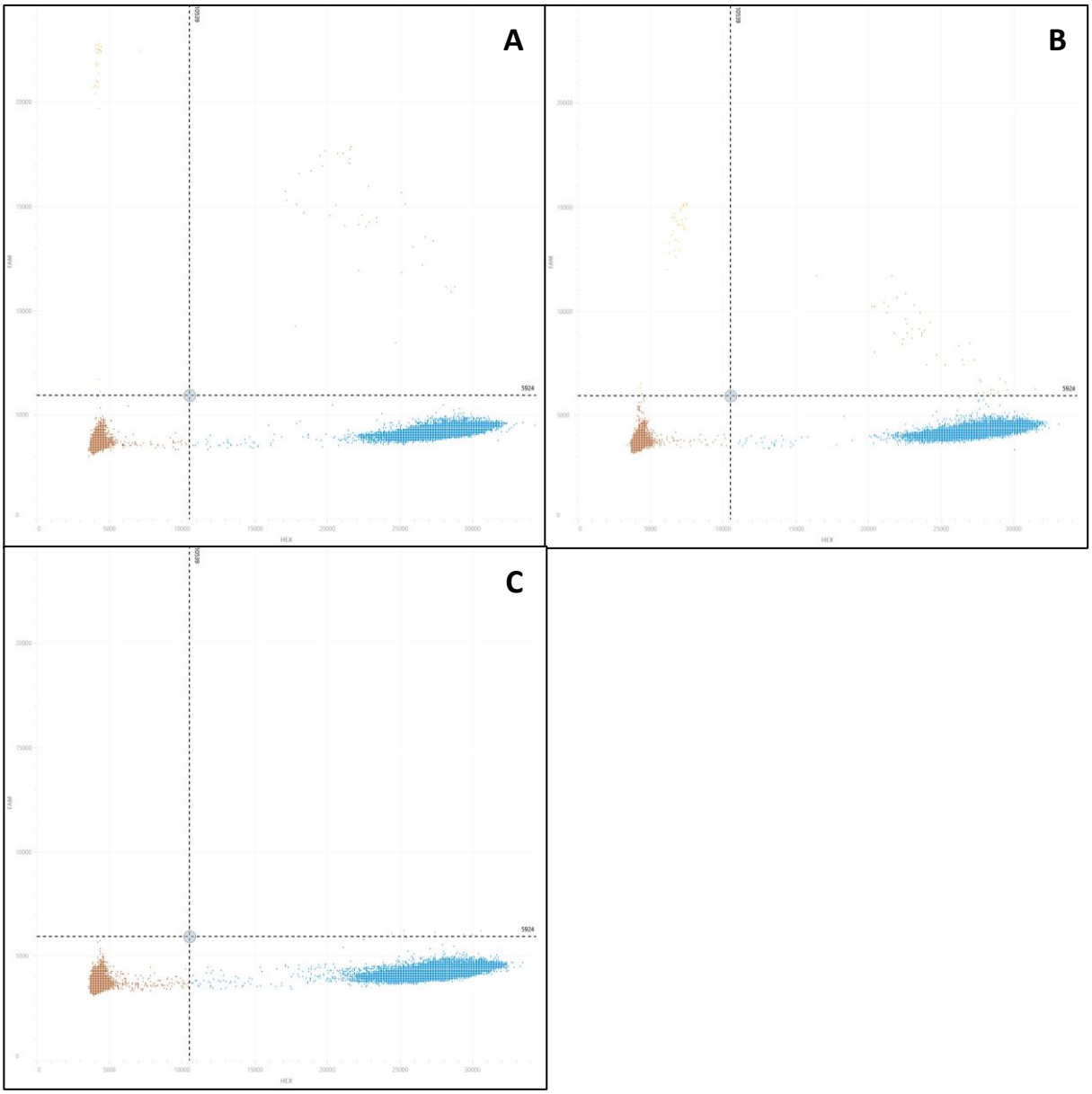

Figure 3. A) 2D scatter plot of a 0.1% G>C mutant template in a 1cpp WT background. **B)** 2D scatter plot of a 0.1% T>A mutant template in a 1cpp WT background. **C)** 2D scatter plot of WT sample (1cpp). Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive.

Version History

Tech Sheet ID	Change/ Event	Date
V1.0	Initial Release	2024-07-24