

Sample Preparation Outline

	<u>FAM (Target)</u>	<u>HEX (Background)</u>	<u>Sample Type</u>
1	100,000	10,000	Positive Control
2	10,000	10,000	Unknown
3	1,000	10,000	Unknown
4	100	10,000	Unknown
5	10	10,000	Unkown
6	1	10,000	Unknown
7	0	10,000	Negative Control
8	0	0	No Template Control

(in copies per 20ul reaction)

Master Mix Preparation

	<u>25ul</u>	<u>x</u>	<u>3 (triplicate)</u>	<u>x</u>	<u>8.5 (column)</u>	<u>637.5ul Final</u>
2X Super	12.5ul					318.75ul
FAM Assay	1.25ul					31.875ul
HEX Assay	1.25ul					31.875ul
						45ul per well

Sample 1

FAM DNA 19ul
 HEX DNA 5ul
 H2O 6ul

Sample 2

FAM DNA 1.9ul
 HEX DNA 5ul
 H2O 23.1ul

Sample 3

FAM DNA Dil 1, 19ul
 HEX DNA 5ul
 H2O 6ul

Sample 4

FAM DNA Dil 1, 1.9ul
HEX DNA 5ul
H2O 23.1ul

Sample 5

FAM DNA Dil 2, 19ul
HEX DNA 5ul
H2O 6ul

Sample 6

FAM DNA Dil 2, 1.9ul
HEX DNA 5ul
H2O 23.1ul

Sample 7

FAM DNA 0ul
HEX DNA 5ul
H2O 25ul

Sample 8

FAM DNA 0ul
HEX DNA 0ul
H2O 30ul

FAM DNA at 20,000 per ul

FAM Dilution 1, 200 per ul (5ul stock + 495ul H2O)

FAM Dilution 2, 2 per ul (5ul of 200 per ul + 495ul H2O)

HEX DNA at 7, 500 per ul