

TECHNICAL BRIEF

AlphaScreen® SureFire® master mixes

2-step protocols, adherent cell

- The suggested volumes assume that you are manually pipetting or using a repeat pipette (no troughs, no automation) in 384-well format. These are recommended master mix recipes when working with adherent cells.
- Please check to make sure that you are using a 2-step kit where the original protocol recommends mixing the Reaction buffer, Activation buffer, and Acceptor beads in a 40:10:1 ratio. This is indicated in the manual, in the "Buffer preparations and Storage Conditions" table. Different kits use different master mix recipes.

Reaction buffer + Activation buffer and AlphaScreen® Acceptor beads	Mix Reaction buffer (40 parts), Activation Buffer (10 parts) and Acceptor beads (1 part). Mix to be stored at room temperature and used the same day, excess mix should be discarded.
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Reaction buffer + Acceptor beads mix:

# of Reactions	Volume Reaction buffer (µL)	Volume Activation buffer (µL)	Volume Acceptor beads (µL)	Prepared mixture volume (µL)	Minimum volume mixture required (µL)
50 Reactions	240	60	6	306	250
100 Reactions	440	110	11	561	500
150 Reactions	640	160	16	816	750
200 Reactions	840	210	21	1071	1000
250 Reactions	1040	260	26	1326	1250
300 Reactions	1240	310	31	1581	1500
400 Reactions	1640	410	41	2091	2000
500 Reactions	2000	500	50	2550	2500

Dilution buffer + Donor beads mix:

# of Reactions	Volume Dilution buffer (μL)	Volume Donor beads (μL)	Prepared mixture volume (μL)	Minimum volume mixture required (μL)
50 Reactions	140	7	147	100
100 Reactions	240	12	252	200
150 Reactions	340	17	357	300
200 Reactions	440	22	462	400
250 Reactions	520	26	546	500
300 Reactions	620	31	651	600
400 Reactions	820	41	861	800
500 Reactions	1000	50	1050	1000