

- 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 <u>45:5:1 ratio</u>, respectively. 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 (45 parts), Activation Buffer (5 parts) and Acceptor beads (1 part). Mix to be stored at room temperature and used the same day, excess mix should be discarded.

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	270	20	6	200	250
Reactions	270	30	6	306	250
100					
Reactions	495	55	11	561	500
150					
Reactions	720	80	16	816	750
200					
Reactions	945	105	21	1071	1000
250					
Reactions	1170	130	26	1326	1250
300					
Reactions	1395	155	31	1581	1500
400					
Reactions	1845	205	41	2091	2000
500					
Reactions	2250	250	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		_		100
Reactions	140	/	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