

### AB 7500 Reaction Components for Murine norovirus (MNV)- crAssphage- IC Assay

Reagent	Stock Concentration	Volume per 25 µl reaction	Final Concentration
Nuclease Free Water		<b>7.68 µl</b>	-
OneStep RT-PCR Buffer	5 X	<b>5.0 µl</b>	1 X
MgCl <sub>2</sub> <sup>A</sup>	50mM	<b>0.75 µl</b>	1.5 mM
dNTP Mix	10 mM	<b>1 µl</b>	0.4 mM
MNVF	10 µM	<b>0.5 µl</b>	0.2 µM
MNVR	10 µM	<b>0.5 µl</b>	0.2 µM
crAssF	10 µM	<b>0.75 µl</b>	0.3 µM
crAssR	10 µM	<b>0.75 µl</b>	0.3 µM
IC46F	10 µM	<b>0.19 µl</b>	0.075 µM
IC194R	10 µM	<b>0.19 µl</b>	0.075 µM
MNVP (Cy5)	10 µM	<b>0.25 µl</b>	0.1 µM
crAssP (JOE)	10 µM	<b>0.63 µl</b>	0.25 µM
ICP (TexasRed)	10 µM	<b>0.38 µl</b>	0.15 µM
OneStep RT-PCR Enzyme	n/a	<b>1.00 µl</b>	n/a
Superase-in	20 Units/µl	<b>0.25 µl</b>	5 Units
FAM ref dye <sup>B</sup>	n/a	<b>2 µl</b>	n/a
Internal Control (IC) <sup>C</sup> RNA	n/a	<b>0.2 µl</b>	n/a
RNA		<b>3 µl</b>	

<sup>A</sup> With the addition of 1.5 mM MgCl, the final concentration per reaction is 4.0 mM.

<sup>B</sup> Use a 1:1000 dilution (made in Primer TE) of FAM reference dye in the N1-N2-IC triplex assay.

<sup>C</sup> Amount varies with concentration of IC RNA. The amount of IC RNA template needs to be adjusted based on the prepared stock concentration to report a Cycle threshold (Ct) of 20-25 when no inhibition is present in the reaction (i.e., the negative control reaction).