

AB 7500 Amplification Reaction Components for SARS-CoV-2 N1-N2-IC Triplex Assay

Reagent	Stock Concentration	Volume per 25 μ l reaction	Final Concentration
Nuclease Free Water		5.93 μl	-
OneStep RT-PCR Buffer	5 X	5.0 μl	1 X
MgCl ₂ ^A	50mM	0.75 μl	1.5 mM
dNTP Mix	10 mM	1 μl	0.4 mM
N1F	10 μ M	1 μl	0.4 μ M
N1R	10 μ M	1 μl	0.4 μ M
N2F	10 μ M	1 μl	0.4 μ M
N2R	10 μ M	1 μl	0.4 μ M
IC46F	10 μ M	0.19 μl	0.075 μ M
IC194R	10 μ M	0.19 μl	0.075 μ M
N1P (JOE)	10 μ M	0.63 μl	0.250 μ M
N2P (Cy5)	10 μ M	0.63 μl	0.250 μ M
ICP (TexasRed)	10 μ M	0.25 μl	0.1 μ M
OneStep RT-PCR Enzyme	n/a	1.00 μl	n/a
Superase-in	20 Units/ μ l	0.25 μl	5 Units
FAM ref dye ^B	n/a	2 μl	n/a
Internal Control (IC) ^C RNA	n/a	0.2 μl	n/a
RNA		3 μl	

^A With the addition of 1.5 mM MgCl₂, the final concentration per reaction is 4.0 mM.

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

^C 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).

AB 7500 Amplification Reaction Components for SARS-CoV-2 N1-IC Duplex Assay

Reagent	Stock Concentration	Volume per 25 μ l reaction	Final Concentration
Nuclease Free Water		8.55 μl	-
OneStep RT-PCR Buffer	5 X	5.0 μl	1 X
MgCl ₂ ^A	50mM	0.75 μl	1.5 mM
dNTP Mix	10 mM	1 μl	0.4 mM
N1F	10 μ M	1 μl	0.4 μ M
N1R	10 μ M	1 μl	0.4 μ M
IC46F	10 μ M	0.19 μl	0.075 μ M
IC194R	10 μ M	0.19 μl	0.075 μ M
N1P (FAM)	10 μ M	0.63 μl	0.250 μ M
ICP (Cy5)	10 μ M	0.25 μl	0.1 μ M
OneStep RT-PCR Enzyme	n/a	1.00 μl	n/a
Superase-in	20 Units/ μ l	0.25 μl	5 Units
ROX ref dye ^B	n/a	2 μl	n/a
Internal Control (IC) ^C RNA	n/a	0.2 μl	n/a
RNA		3 μl	

^A With the addition of 1.5 mM MgCl, the final concentration per reaction is 4.0 mM.

^B Use a 1:10 dilution (made in Primer TE) of ROX reference dye in the N1-IC duplex assay.

^C 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).

AB 7500 Amplification Reaction Components for SARS-CoV-2 N2-IC Duplex Assay

Reagent	Stock Concentration	Volume per 25 µl reaction	Final Concentration
Nuclease Free Water		8.55 µl	-
OneStep RT-PCR Buffer	5 X	5.0 µl	1 X
MgCl ₂ ^A	50mM	0.75 µl	1.5 mM
dNTP Mix	10 mM	1 µl	0.4 mM
N2F	10 µM	1 µl	0.4 µM
N2R	10 µM	1 µl	0.4 µM
IC46F	10 µM	0.19 µl	0.075 µM
IC194R	10 µM	0.19 µl	0.075 µM
N2P (FAM)	10 µM	0.63 µl	0.250 µM
ICP (Cy5)	10 µM	0.25 µl	0.1 µM
OneStep RT-PCR Enzyme	n/a	1.00 µl	n/a
Superase-in	20 Units/µl	0.25 µl	5 Units
ROX ref dye ^B	n/a	2 µl	n/a
Internal Control (IC) ^C RNA	n/a	0.2 µl	n/a
RNA		3 µl	

^A With the addition of 1.5 mM MgCl, the final concentration per reaction is 4.0 mM.

^B Use a 1:10 dilution (made in Primer TE) of ROX reference dye in the N1-IC duplex assay.

^C 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).