

HT Liquid Mock Reagents

Description

The HT Liquid Mock Reagents simulate high throughput (HT) reagents found in the FUSIONPlex™-HT and(or) VARIANTPlex™-HT kits. HT Liquid Mock Reagents are intended for the optimization and training of liquid class performance on automated liquid handling platforms. These reagents are not intended to be used for library preparation.

Before Getting Started

Materials supplied

Mock VARIANTPlex-HT for Illumina – 24 reactions (dSK0296)

Store at -30°C to -10°C

Component Description	Part Number
<i>DNA Fragmentation Buffer Mix</i>	SA0838
<i>Complete End Repair Buffer Mix</i>	SA0804
<i>Ligation Step 1 Buffer Mix</i>	SA0807
<i>Ligation Step 2 Buffer Mix 2.0</i>	SA0810
<i>First PCR Buffer Mix 2.0</i>	SA0834
<i>Second PCR Buffer Mix 2.0</i>	SA0835

Mock FUSIONPlex-HT for Illumina – 24 reactions (dSK0295)

Store at -30°C to -10°C

Component Description	Part Number
<i>Random Priming Buffer Mix</i>	SA0826
<i>First Strand cDNA Synthesis Buffer Mix</i>	SA0827
<i>Second Strand Synthesis Buffer Mix</i>	SA0830
<i>Complete End Repair Buffer Mix</i>	SA0804
<i>Ligation Step 1 Buffer Mix</i>	SA0807
<i>Ligation Step 2 Buffer Mix 2.0</i>	SA0810
<i>First PCR Buffer Mix 2.0</i>	SA0834
<i>Second PCR Buffer Mix 2.0</i>	SA0835

Library Prep Cleanup Reagents – 24 reactions (SK0190)

Store at 2°C to 8°C

Component Description	Part Number
<i>Ligation Cleanup Beads</i>	SA0655
<i>Ligation Cleanup Buffer</i>	SA0656

Materials required but not supplied

Description	Supplier	Part Number
<i>1 M Tris-HCl, pH 8.0 (molecular biology grade)</i>	<i>Various</i>	-
<i>Ultrapure water (molecular biology grade)</i>	<i>Various</i>	-
<i>glycerol</i>	<i>Various</i>	

Reagents to prepare before starting

- Make at least 1 mL of 50% glycerol from 100% glycerol stock.
 - Mix **500 µL** 100% glycerol with **500 µL** ultrapure water.
 - 50% glycerol can be used for up to one week after mixing.
- Make at least **10 mL** fresh 10 mM Tris-HCl, pH 8.0 from 1 M Tris-HCl, pH 8.0 and ultrapure water.
 - Mix **100 µL** 1M Tris-HCl, pH 8.0 with **9,900 µL** ultrapure water.
 - 10 mM Tris-HCl, pH 8.0 can be used for up to one week after mixing.

Master Mix Instructions

Mock master mix formulations are provided for reference but may not be applicable to the workflow being automated. See page 4 for FUSION[™]lex-HT protocol for Illumina and page 8 for VARIANT[™]lex-HT Protocol for Illumina. **NOTE: The volume of 10 mM Tris-HCl, pH 8.0 indicated in these instructions is intended to mimic sample addition. This addition will result in a solution representing the final reaction volume and consistency. If the intended purpose is to mimic only the master mix, 10 mM Tris-HCl, pH 8.0 should not be added.**

RA-DOC-049, FUSION^{Pro}lex-HT Protocol for Illumina

Step 1: Random Priming

Random Priming Mock Master Mix	1X (μL)	___X (μL)
Random Priming Buffer Mix (SA0826)	3	
10 mM Tris-HCl, pH 8.0	12	
<i>Total volume</i>	15	

Step 2: First Strand cDNA Synthesis

First Strand cDNA Synthesis Mock Master Mix	1X (μL)	___X (μL)
First Strand cDNA Synthesis Buffer Mix (SA0827)	3	
50% Glycerol	2	
10 mM Tris-HCl, pH 8.0	15	
<i>Total volume</i>	20	

Step 3: Second Strand cDNA Synthesis

Second Strand Synthesis Mock Master Mix	1X (μL)	___X (μL)
Second Strand Synthesis Buffer Mix (SA0830)	15	
50% Glycerol	5	
Nuclease-free water	1	
10 mM Tris-HCl, pH 8.0	19	
<i>Total Volume</i>	40	

Step 5: Complete End Repair

Complete End Repair Mock Master Mix	1X (μL)	___X (μL)
Complete End Repair Buffer Mix (SA0804)	8.86	
50% Glycerol	1.14	
10 mM Tris-HCl, pH 8.0	40	
<i>Total Volume</i>	50	

Step 6: Ligation Step 1

Ligation Step 1 Mock Master Mix	1X (μL)	___X (μL)
Ligation Step 1 Buffer Mix (SA0807)	3.7	
50% Glycerol	0.3	
10 mM Tris-HCl, pH 8.0	16	
<i>Total Volume</i>	20	

Step 8: Ligation Step 2

Ligation Step 2 Mock Master Mix	1X (μL)	___X (μL)
Ligation Step 2 Buffer Mix 2.0 (SA0810)	24.6	
50% Glycerol	0.4	
10 mM Tris-HCl, pH 8.0	25	
<i>Total volume</i>	50	

Step 9: First PCR

First PCR Mock Master Mix	1X (μL)	___X (μL)
First PCR Buffer Mix 2.0 (SA0834)	11.2	
50% Glycerol	0.8	
10 mM Tris-HCl, pH 8.0	28	
<i>Total volume</i>	40	

Step 10: Second PCR

Second PCR Mock Master Mix	1X (μL)	__X (μL)
Second PCR Buffer Mix 2.0 (SA0835)	11.2	
50% Glycerol	0.8	
10 mM Tris-HCl, pH 8.0	28	
<i>Total volume</i>	<i>40</i>	

RA-DOC-058, VARIANT*Plex*-HT Protocol for Illumina

Step 1: DNA Fragmentation

DNA Fragmentation Mock Master Mix	1X (μL)	___X (μL)
DNA Fragmentation Buffer Mix (SA0838)	3.2	
50% Glycerol	4.8	
10 mM Tris-HCl, pH 8.0	32	
<i>Total volume</i>	<i>40</i>	

Step 2: Complete End Repair

Complete End Repair Mock Master Mix	1X (μL)	___X (μL)
Complete End Repair Buffer Mix (SA0804)	8.86	
50% Glycerol	1.14	
10 mM Tris-HCl, pH 8.0	40	
<i>Total Volume</i>	<i>50</i>	

Step 3: Ligation Step 1

Ligation Step 1 Mock Master Mix	1X (μL)	___X (μL)
Ligation Step 1 Buffer Mix (SA0807)	3.7	
50% Glycerol	0.3	
10 mM Tris-HCl, pH 8.0	16	
<i>Total Volume</i>	20	

Step 5: Ligation Step 2

Ligation Step 2 Mock Master Mix	1X (μL)	___X (μL)
Ligation Step 2 Buffer Mix 2.0 (SA0810)	24.6	
50% Glycerol	0.4	
10 mM Tris-HCl, pH 8.0	25	
<i>Total volume</i>	50	

Step 6: First PCR

First PCR Mock Master Mix	1X (μL)	___X (μL)
First PCR Buffer Mix 2.0 (SA0834)	11.2	
50% Glycerol	0.8	
10 mM Tris-HCl, pH 8.0	28	
<i>Total volume</i>	40	

Step 7: Second PCR

Second PCR Mock Master Mix	1X (μL)	___X (μL)
Second PCR Buffer Mix 2.0 (SA0835)	11.2	
50% Glycerol	0.8	
10 mM Tris-HCl, pH 8.0	28	
<i>Total volume</i>	<i>40</i>	

Limitations of use

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Revision History

Document Number	Date	Description of change
<i>RA-DOC-465/REV01</i>	October 2023	Initial release.
<i>RA-DOC-465/REV02</i>	November 2023	Updated branding.