sageHLS™
HMW Library System

Guidelines for Preparing HLS-CATCH Enzyme Mix

A. Reagents: Supplied by User

The HLS-CATCH system has been tested and validated using the following reagents and suppliers:

S.pyogenes Cas9 enzyme, wild Type:

New England Biolabs		
M0386T	400 pmol	20 µM
M0386M	2,000 pmol	20 µM

Guide RNAs:

Integrated DNA Technologies (IDT)
These are provided in two halves; crRNA and tracRNA, and must be annealed before they will assemble with the S.pyogenes Cas9 enzyme. The crRNAs must be designed to flank the target sequence, and the tracRNA is identical for all gRNAs.

B. Preparation: 30 minutes

The HLS-CATCH enzyme mix can be prepared prior to starting the HLS Workflow, or during the Extraction Stage of the Workflow which typically requires 1 hour. If the enzyme mix is prepared ahead of time and placed on ice, it will remain stable for several hours.

1. Anneal the tracRNA and crRNAs

a. Add equimolar concentrations of tracRNA and crRNAs. Stocks are dissolved in IDT Duplex Buffer (30mM HEPES, pH7.5, 100 mM Potassium Acetate):

	vol. µl	stock [ ] µM	final [ ] in anneal mixture µM
tracRNA	20	100	50
crRNA1	10	100	25
crRNA2	10	100	25
TOTAL	40		

b. Heat the mix at 95oC for 5 minutes (in a heating block or thermal cycler)

c. Remove the mix from heat, and allow to cool on the bench-top

In some cases it might be useful to use gRNAs which cut at closely spaced sites on either side of the target sequence. In this instance, anneal with equimolar amounts of each crRNA, with the total moles of crRNA equal to the total moles of tracRNA. If designing a system with two cut sites on either side of the target, anneal with all four crRNAs at 12.5μM and tracRNA at 50μM.

2. Assemble the Cas9-gRNA complete reaction mixture

a. Using the following order of addition, assemble the Cas9-gRNA reaction mixture:

order of addtion	vol. μl	stock [ ] μM	final [ ] in enzyme mixture μM	
1	16			**Nuclease free H2O**
2	20	4	1	**4X Enzyme Buffer (provided by Sage Science*)**
3	40	50	25	**Annealed gRNA mix**
4	4	20	1	**NEB Cas9 nuclease, wt**
TOTAL	80			

b. Mix by pipetting up and down.

c. Incubate at 37oC for 10 minutes in a thermal cycler or heat block.

d. The Enzyme Mix can be placed on ice for up to 3 hours prior to use.

*** provided with the HIT-0004 and HIT-0012 Cassette Kits.**