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CasX Cleavage Assay

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1 Works for me dx.doi.org/10.17504/protocols.io.8wshxee

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ABSTRACT

This protocol describes a CasX Cleavage Assay.

CasX_Cleavage_Assay_Protocol.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Magnesium Chloride	AC223210010	Fisher Scientific
HEPES	H6147	Sigma Aldrich
NaCl	S-3014	Sigma-aldrich
DEPC (Diethyl pyrocarbonate)	DB0154.SIZE.5ml	Bio Basic Inc.
Potassium Chloride	P9541	Sigma Aldrich
Glycerol	G5516	Sigma Aldrich
EDTA	17892	Thermo Fisher
Tris Hydrochloride (Tris-HCl)	RES3098T-B7	Sigma Aldrich
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)	C4706	Sigma Aldrich
Heparin sodium	H0200000	Sigma Aldrich

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Either purchase ribonucleoprotein from a vendor or express and purify the protein beforehand.

Prepare Buffers and Solutions

1 Prepare *CasX reaction buffer*.

1.1

Mix together [M]20 Milimolar (mM) **HEPES (pH 7.5)** , [M]10 Milimolar (mM) **magnesium chloride** , [M]150 Milimolar (mM) **potassium chloride** , [M]1 % volume **glycerol** , and [M]0.5 Milimolar (mM) **TCEP** .

2 Prepare *CasX dilution buffer*.

2.1

Mix together [M]500 Milimolar (mM) **NaCl** , [M]10 % volume **glycerol** , [M]20 Milimolar (mM) **Tris-HCl (pH 7.5)** , [M]1 Milimolar (mM) **magnesium chloride** , and [M]0.5 Milimolar (mM) **TCEP** .

3 Prepare *quencher*.

3.1

Mix together [M]0.5 undefined **heparin** and [M]25 Milimolar (mM) **EDTA** .

4 Prepare Formamide Loading Dye (2x concentrated).

4.1 Add 20 ml **formamide** .

4.2 Add EDTA to a final concentration of [M]10 Milimolar (mM) (2.2 ml of [M]100 Milimolar (mM) stock).

4.3 Add a spatula tip-ful of powdered xylene cyanol.

4.4 Add a spatula tip-ful of powdered bromophenol blue.

Annealing Duplex DNA

5 Prepare stock of labelled target strand DNA to be 100 µl of [M]100 Nanomolar (nM) .

6

Mix **1:1.2 molar ratio** of ^{32}P labelled target strand (TS) to unlabelled nontarget strand (NTS) of duplex substrate:

Stock of unlabelled TS at 50 µl , [M]120 Nanomolar (nM) : dilute 4.48 µl TS stock in 45.42 µl DEPC.

Stock of unlabelled NTS at 50 µl , [M]120 Nanomolar (nM) : dilute 3.56 µl TS stock in 46.44 µl DEPC.

7

Mix 50 µl of [M]100 Nanomolar (nM) labelled target strand with 50 µl of [M]120 Nanomolar (nM) unlabelled nontarget strand to make a 100 µl stock of duplex substrate.

Reverse concentrations for labelled nontarget, unlabelled target.

8

Place on 95 °C heat block for 00:02:00 - 00:05:00 , then slow cool for about 02:00:00 . This is the **Duplex DNA stock** for cleavage assays.

DNA stock was diluted to 120nM with CasX reaction buffer.

CasX Activity Assays

9 Dilute CasX to [M]4 Micromolar (µM) in *CasX dilution buffer* (components shown above in go to step #2).

10 Dilute sgRNA to [M]6 Micromolar (µM) in *CasX reaction buffer* (components shown above in go to step #1).

11

Mix an equimolar ratio of CasX and sgRNA (2 µl sgRNA stock, 3 µl CasX stock, 1 µl *CasX reaction buffer*).

12 Allow samples to mix for 00:30:00 at Room temperature .

13 Dilute annealed, labelled duplex DNA into *CasX reaction buffer* (2 µl Duplex DNA stock, 43 µl *reaction buffer*).

14 Add RNP stock (5 µl , [M]2 Micromolar (µM) RNP) to the DNA:buffer mixture. Total volume should now be 50 µl.

15

Incubate at 37 °C . For each timepoint (0, 1, 5, 10, 30, 60, 120 minutes), remove 5 µl and add to a tube filled with 5 µl *quencher* (CasX mixture and quencher solution should have equal volumes).

16 Incubate with *quencher* solution at **Room temperature** for **00:05:00** .

17 Add **5 µl** formamide loading dye and incubate at **95 °C** for **00:05:00** .

18 After quenching, load samples (4 µl/well, i.e. approximately 200 cpm/well was loaded per sample) on a 12 % PAGE gel and run at 40 – 45 W for **00:45:00** .

Large gel was pre-warmed at 25W for ~45minutes.

Loading less (50-100cpm/well) sample works fine. You may need to expose overnight.