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 We use this protocol and it's working

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CasX Cleavage Assay

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ABSTRACT

This protocol describes a CasX Cleavage Assay.

ATTACHMENTS

[CasX_Cleavage_Assay_Protocol.pdf](#)

MATERIALS

MATERIALS

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

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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 millimolar (mM) HEPES (pH 7.5) ,

[M] 10 millimolar (mM) magnesium chloride , [M] 150 millimolar (mM) potassium chloride ,

[M] 1 % volume glycerol , and [M] 0.5 millimolar (mM) TCEP .

2 Prepare *CasX* dilution buffer.

2.1

Mix together [M] 500 millimolar (mM) NaCl , [M] 10 % volume glycerol ,

[M] 20 millimolar (mM) Tris-HCl (pH 7.5) , [M] 1 millimolar (mM) magnesium chloride , and

[M] 0.5 millimolar (mM) TCEP .

3 Prepare *quencher*.

3.1

Mix together [M] 0.5 Mass Percent heparin and [M] 25 millimolar (mM) EDTA .

4 Prepare Formamide Loading Dye (2x concentrated).

- 4.1 Add 20 mL formamide .
- 4.2 Add EDTA to a final concentration of 10 millimolar (mM) (2.2 mL of 100 millimolar (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 μ L of 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 μ L , 120 nanomolar (nM) : dilute 4.48 μ L TS stock in 45.42 μ L DEPC.
Stock of unlabelled NTS at 50 μ L , 120 nanomolar (nM) : dilute 3.56 μ L TS stock in 46.44 μ L DEPC.
- 7 Mix 50 μ L of 100 nanomolar (nM) labelled target strand with 50 μ L of 120 nanomolar (nM) unlabelled nontarget strand to make a 100 μ L stock of duplex substrate.

Note

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.

Note

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

CasX Activity Assays

9

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

10

Dilute sgRNA to 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 .

Note

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

Note

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