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CasX Cleavage Assay

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1 Works for me

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ABSTRACT

This protocol describes a CasX Cleavage Assay.

CasX_Cleavage_Assay_Pro tocol.pdf

MATERIALS

NAME Y	CATALOG #	VENDOR V
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-HCI)	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

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.

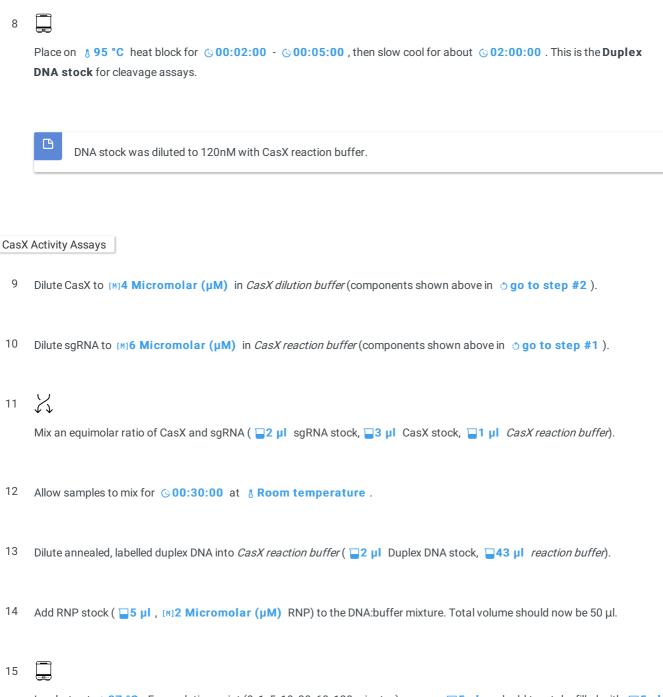
Annealing Duplex DNA

- 5 Prepare stock of labelled target strand DNA to be $\boxed{100 \ \mu}$ of $\boxed{100 \ Nanomolar (nM)}$.
- 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 $\Box 50~\mu I$, [M]120 Nanomolar (nM) : dilute $\Box 4.48~\mu I$ TS stock in $\Box 45.42~\mu I$ DEPC. Stock of unlabelled NTS at $\Box 50~\mu I$, [M]120 Nanomolar (nM) : dilute $\Box 3.56~\mu I$ TS stock in $\Box 46.44~\mu I$ DEPC.

7	Mix 350 μl of [M]100 Nanomolar (nM) nontarget strand to make a 100 μl stock		ıl of [M]120 Nanomolar (nM) unlabelled
	Reverse concentrations for labelled	nontarget, unlabelled target.	



16	
	Incubate with $\it quencher$ solution at $\it 8$ Room temperature for $\it \odot$ 00:05:00 .
17	Add $\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $
18	After quenching, load samples (4 μ l/well, i.e. approximately 200 cpm/well was loaded per sample) on a 12 % PAGE gel and run at 40 – 45 W for \odot 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.
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