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DNA/RNA Radiolabeling Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.8dshs6e

The Center for Genome Editing and Recording

Meredith Triplet

Radiolabeling_CasX_DNA_substrates.pdf

GUIDELINES

CasX TS/NTS with non-hydrolysable spacers:

TS:

5'-CGCTAGCTACG**T*T*T*G*A*T*T*T*T*C*T*G*C*T*G*C*A*G*G*A*** *TGAA*ATCCCGTAATCGCGC-3'

MW: 15664.2 g/mol
Concentration: X µM

***= phosphothioate, **bold letters** = PAM, *italic letters* = spacer

For 10 pmol of TS: X µl of stock

NTS:

5'-GCGCGATTACGGGAT*TTCA***T*C*C*T*G*C*A*G*C*A*G*A*A*A*A*T*C*A*A*A***A*CGTAGCTAGCG-3'

MW: 15749.3 g/mol
Concentration: X µM

***= phosphothioate, **bold letters** = PAM, *italic letters* = spacer

For 10 pmol of NTS: X µl of substrate

Labelling reaction setup:

*TS:

XX ulDNA or RNA (10 pmoles)
2.5 ul10x PNK buffer
0.5 ulPNK enzyme
1.5 ulP32-gamma-ATP
XX mL dH2O (DEPC for labeling RNA) to 25 ul

*NTS:

XX ulDNA or RNA (10 pmoles)
2.5 ul10x PNK buffer
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XX mL dH2O (DEPC for labeling RNA) to 25 ul

MATERIALS

NAME	CATALOG #	VENDOR
T4 Polynucleotide Kinase (3' phosphatase minus) - 200 units	M0236S	New England Biolabs
10X T4 PNK Reaction Buffer		New England Biolabs
ATP [γ-32P]- 3000Ci/mmol 10mCi/ml Lead 100 µCi (P32-gamma-ATP)	NEG002A100UC	Perkin Elmer
HiTrap Desalting columns with Sephadex G-25 resin	29048684	Ge Life Sciences

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Set up labeling reaction:

X µl	DNA or RNA (10 pmoles)
2.5 µl	10x PNK buffer
0.5 µl	PNK enzyme
1.5 µl	P32-gamma-ATP
	dH2O (DEPC for labeling RNA) to 25 µl

Mix the DNA, buffer, enzyme, and H_2O at the bench, and then add the DNA/enzyme mixture to ATP-filled tubes in a radioactive use area.

2

Incubate at **37 °C** for **00:30:00** .

3 Heat inactivate the PNK at **65 °C** for **00:20:00** .

4

Prepare G25 columns (from GE, green box): vortex thoroughly, twist cap ¼ turn, snap off bottom, spin for **00:01:00** at **3000 rpm** to get rid of liquid.

5 

Add **50 µl** H_2O to a labeled eppendorf tube, place G25 column in it.

6 

Add **25 µl** H_2O to each labeling reaction after heat inactivation is done.

7 Apply entire reaction (now 50 µl total) to G25 column resin.

8 

Spin for **00:02:00** at **3000 rpm** .

9 Since 50 µl H_2O were in bottom of tube and you add your 50 µl reaction, you should end with up to 100 µl of **100 Nanomolar (nM)** labeled DNA/RNA.

10 

Measure **1 µl** of each reaction with the black rad counter on shelf to get cpm readings.