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DNA/RNA Radiolabeling Protocol

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Protocol status: Working
We use this protocol and it's working

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Keywords: Radiolabeling, DNA, RNA, CasX, TS, NTS

Attachments

PDF

Radiolabeling_CasX_D...

141KB

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**∗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
0.5 ulPNK enzyme
1.5 ulP32-gamma-ATP
XX mL dH2O (DEPC for labeling RNA) to 25 ul

Materials

MATERIALS

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

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

Note

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 [M] 100 nanomolar (nM) labeled DNA/RNA.

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