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

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DNA/RNA Radiolabeling Protocol

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ATTACHMENTS

[Radiolabeling_CasX_DNA_substrates.pdf](#)

GUIDELINES

CasX TS/NTS with non-hydrolysable spacers:

TS:

5'-
 CGCTAGCTACGT***T*****T*****G*****A*****T*****T*****T*****C*****T*****G*****C*****T*****G*****C*****A*****G*****G*****A*** TGAAATCCC
 GTAATCGCGC-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 *TTCAT****C*****C*****T*****G*****C*****A*****G*****C*****A*****G*****A*****A*****A*****T*****C*****A*****A*****A*****C**
 GTAGCTAGCG-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 µl DNA or RNA (10 pmoles)
2.5 µl 10x PNK buffer
0.5 µl PNK enzyme
1.5 µl P32-gamma-ATP
XX mL dH₂O (DEPC for labeling RNA) to 25 µl

***NTS:**

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

MATERIALS

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⊗ 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 Life Sciences Catalog #29048684**

SAFETY WARNINGS

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

1 Set up labeling reaction:

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

Note

Mix the DNA, buffer, enzyme, and H₂O 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 μ L H₂O 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.