



Nov 21,
2019

DNA/RNA Radiolabeling Protocol

Liz O'Brien¹, Connor Tsuchida¹

¹University of California, Berkeley

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'-CGCTAGCTACGT***T*****T*****G*****A*****T*****T*****T*****C*****T*****G*****C*****T*****G*****C*****A*****G*****G*****A*** *TGAAATCCCGTAATCGCGC*-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 *TT*CAT***C*****C*****T*****G*****C*****A*****G*****C*****A*****G*****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 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

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 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 µl** H₂O to a labeled eppendorf tube, place G25 column in it.


6 

Add  **25 µl** H₂O to each labeling reaction after heat inactivation is done.

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

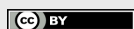
8 

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

9 Since 50 µl H₂O 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.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited