

pA-Tn5 Loading Protocol for Active Motif CUT&Tag-IT™ Assays

Recipe for 1X pA-Tn5 Transposome Storage Solution:

Chemical	Final Conc.
Glycerol (Molecular Biology), density 1.28 g/mL	50% (v/v)
Sodium chloride (NaCl) 5 M CAS 7647-14-5	200 mM
HEPES, 1 M, pH 7.5 CAS 7365-45-9	100 mM
Dithiothreitol (DTT), CAS 3483-12-3	2.0 mM
Triton X-100 CAS 9002-93-1	0.2%
EDTA 0.5 M, pH 8 CAS 6381-92-6	0.2 mM
Potassium Hydroxide CAS 1310-58-3	pH to 7.2
Water, Molecular Biology Grade	

Store 1X pA-Tn5 Transposome Storage Solution at room temperature.

- Oligo A: 5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG
- Oligo B: 5' G TCT CGT GGG CTC GGA GAT GTG TAT AAG AGA CAG
- Mosaic End Reverse Oligo: 5' PO4-CTG TCT CTT ATA CAC ATC T

For Research Use Only. Not for use in diagnostic procedures.

pA-Tn5 Loading Protocol

Final formulation of pA-Tn5 in the loaded CUT&Tag-IT™ Assay Assembled Transposomes:

Chemical	Final Conc.
Recombinant pA-Tn5 Transposase, bulk (Concentration will be on tube/paperwork – typically 0.3-0.4 mg/mL) MW 72.7 kDa	4.25 - 5.5 μ M (dependent on starting concentration)
1X Transposome Storage Solution	1X
Oligo A, 100 μ M	6.0 μ M
Oligo B, 100 μ M	6.0 μ M
Mosaic End Reverse Oligo, 100 μ M	6.0 μ M

Make Mosaic End Adapter Mixes A and B (note oligos are resuspended in TE):

Anneal Oligo A

1. Mix equal volumes of Oligo A (100 μ M) with Mosaic End Reverse Oligo (100 μ M).
2. Heat in a thermal cycler (95°C for 3 minutes, 65°C for 5 minutes, cool to 10°C (with a ramp rate of 0.1°C/second for all cooling steps).

Anneal Oligo B

1. Mix equal volumes of Oligo B (100 μ M) with Mosaic End Reverse Oligo (100 μ M).
2. Heat in a thermal cycler (95°C for 3 minutes, 65°C for 5 minutes, cool to 10°C (with a ramp rate of 0.1°C/second for all cooling steps).

Prepare CUT&Tag-IT Assay Assembled Transposomes (pA-Tn5 MW 72.7 kDa)

1. In one tube, combine pA-Tn5 in a 1:1 molar ratio with annealed primers.
For example, final concentration of 8 μ M pA-Tn5, 4 μ M Annealed Oligo A, and 4 μ M Annealed Oligo B.
2. Vortex briefly (~1 second) on a low power setting or flick tube gently to mix.
3. Leave at room temperature for 50 minutes.
4. Store at -20°C.

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Technical Support

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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