

Tn5 Loading Protocol for Active Motif ATAC-Seq

Recipe for 1X 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	0.1%
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 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.

Tn5 Loading Protocol

Final formulation of Tn5 in the loaded Assembled Transposomes:

Chemical	Final Conc.
Recombinant Tn5 Transposase, bulk (Concentration will be on tube/paperwork – typically 0.3-0.5mg/mL) MW 53.3 kDa	1 μ M
Oligo A, 100 μ M	0.625 μ M
Oligo B, 100 μ M	0.625 μ M
Mosaic End Reverse Oligo, 100 μ M	1.25 μ M

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

1. Combine Oligo A-Mosaic End Reverse Oligo equal volumes of 100 μ M each oligo.
2. Combine Oligo B-Mosaic End Reverse Oligo equal volumes of 100 μ M each oligo.
3. Place tubes in a 95°C thermocycler with heated lid for 3 minutes.
4. Allow the mixed oligos to come to room temperature in the block without opening the lid, about 30 minutes.
5. Remove from the block and allow to incubate at room temperature for an additional 30 minutes.
6. Pool the contents of the Mosaic End Adapter Mixes together. Mix gently by pipetting.

Prepare Assembled Transposomes (Tn5 MW 53.3 kDa)

7. In one tube, combine Tn5 in a 0.8 μ M Tn5 per 1 μ M molar ratio with Mosaic End Adapter Mix (0.5 μ M of each adapter).
8. Incubate for 1 hour.
9. Dilute to 1 μ M in 1X Transposome Storage Buffer.
10. Mix gently by flicking tube or brief gentle vortex on low power setting.
11. 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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