pA-TN5 transposase (loaded)

Cat. No. C01070001
Format: 15 µl (32 rxns) / 30 µl (64 rxns)
Molecular weight: 67 kD

Product description

pA-Tn5 Transposase is a fusion protein of hyperactive Tn5 transposase and protein A developed for the **CUT&Tag** assay. For ease of use, the fusion protein is pre-loaded with sequencing adapters suitable for single or dual indexing in single or paired-end Illumina platforms. The adaptors contain 19-mer Tn5 mosaic ends and the sequences for PCR amplification with barcoded i7/i5.

Mosaic end_reverse: [PHO]CTGTCTCTTATACACATCT

Mosaic end_Adapter A: **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG

Mosaic end_Adapter B: **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG

Underlined regions correspond to the double-stranded part of the adapter, recognized by the transposase. **Bold regions** represent sequences required per PCR amplification with barcoded i7/i5 primers as described by Buenrostro et al (2015).

The protein A has a high affinity to rabbit polyclonal antibodies, mouse IgG2a, IgG2b and IgA, guinea pig IgG, dog IgG, pig IgG. However, the use of secondary antibody (e.g. guinea pig anti-rabbit) is recommended for a higher sensitivity of **CUT&Tag** assay.

Suggested dilution:

For the standard **CUT&Tag assay**, the recommended dilution of **pA-Tn5 Transposase (loaded)** is 1:250 (0.4 µl of pA-Tn5 for 100 µl of buffer). Please note that depending on the starting amount of cells and/or primary antibody, different dilution in a range 1:50-1:500 might be tested.

Storage conditions

Store at -20°C.

Storage buffer

Supplied in solution containing 50% v/v glycerol.

Precautions

This product is for research use only. Not for use in diagnostic or therapeutic procedures.

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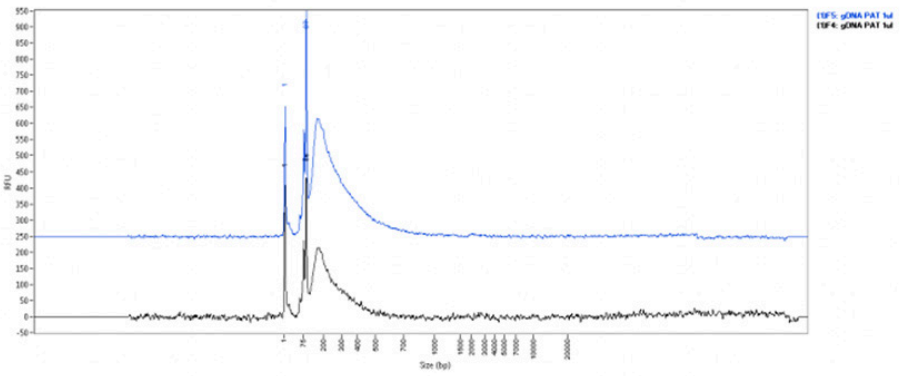
Last update: August, 2022

Quality control

Each lot of pA-Tn5 transposase is quality checked by an in vitro activity test (cleavage of human genomic DNA) (Figure 1, A) and by a CUT&Tag assay using H3K27me3 polyclonal ChIP-seq grade antibody (Cat. No. C15410195) (Figure 1, B).

A.



B.

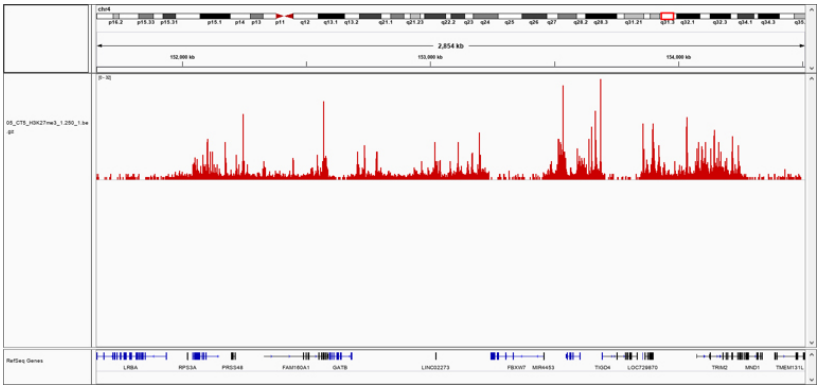


Figure 1: Quality control of pA-Tn5 transposase loaded with sequencing adapters

A: The Fragment Analyzer trace showing the representative cleavage pattern of gDNA. The pA-Tn5 fusion protein (Cat. No. C01070001) efficiently digests gDNA to a smear. 500 ng of human genomic DNA were incubated for 7 min at 55°C with 1 µl of pA-Tn5 fusion protein loaded with appropriated adaptors in a tagmentation buffer (40mM Tris-HCl pH7.5, 40mM $MgCl_2$ and 12.5% DMF). The reaction was stopped by adding SDS, cleaned-up and resolved on the Fragment Analyzer to assess the cleavage.

B: Representative screenshot at selected locus obtained using Diagenode pA-Tn5 fusion protein (Cat. No. C01070001) and H3K27me3 polyclonal ChIP-seq grade antibody (Cat. No. C15410195) following CUT&Tag protocol (Kaya-Okur, H.S., Nat Commun 10, 1930 (2019)).

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