

Tagmentase (Tn5 transposase) - loaded

Cat. No. **C01070012**

Lot: 4/aD

Format: 10 µl / 30 µl

Description

Diagenode Tagmentase – loaded is a hyperactive Tn5 transposase preloaded with Illumina-compatible sequencing adapters. Its ability to cut DNA and insert the sequencing adapters in one step makes it the perfect companion for Next-Generation Sequencing experiments using powerful technologies such as ATAC-seq or ChIPmentation.

The Tagmentase is pre-loaded with Nextera sequencing adapters compatible with Illumina platforms, as described in figure 1 (based on Picelli et al., 2014). The oligos loaded on the Tagmentase are recognized by Diagenode Primer indexes for Tagmented Libraries to perform single-indexing (Cat. No. C01011033 or C01011032) or unique dual-indexing (Cat. No. C01011035 or C01011034) of the libraries.

Mosaic end_reverse: 5' [PHO]CTGTCTCTTATACACATCT 3'

Mosaic end_Adapter A: 5' TCGTCGGCAGCGTCCAGATGTGTATAAGAGACAG 3'

Mosaic end_Adapter B: 5' GTCTCGTGGGCTCGGGAGATGTGTATAAGAGACAG 3'

Figure 1. Sequences of the oligos used to load the Tagmentase.

Underlined regions correspond to the double-stranded part of the adapter, recognized by the tagmentase.

Storage conditions: Store at -20°C. Guaranteed stable for 6 months from date of receipt when stored properly.

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.

Diagenode sa. BELGIUM | EUROPE

LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA

400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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Examples of use:

Diagenode Tagmentase – loaded can be used in combination with Diagenode Tagmentation Buffer 1x (Cat. No. C01019042) or 2x (Cat. No. C01019043) to perform the tagmentation step in the following protocols:

Fragmentation assay experiments:

- A fragmentation assay can be performed on lambda DNA, using the following incubation mix for 1 reaction:

Tagmentation Buffer (1x)	17 µl
lambda DNA (50 ng/ µl)	2 µl
Tagmentase loaded	1 µl

- The reaction is then incubated 7 minutes at 55°C
- The tagmentation can be stopped by addition of a SDS solution (0.2% final) for 5 minutes incubation at room temperature
- The DNA can then be analyzed on agarose gel

ChIPmentation experiments:

- After Immunoprecipitation, the magnetic beads binding the antibody/chromatin complex are washed and can then be incubated with the following mix for 1 tagmentation reaction:

Tagmentation Buffer (1x)	29 µl
Tagmentase loaded	1 µl
Beads/antibody/chromatin/complex*	

** The quantity of chromatin per reaction will depend on the ChIPmentation experimental design. Successful tagmentation with the proposed protocol has been performed on chromatin from 5,000 to 4,000,000 cells per reaction.*

- The reaction is then incubated 10 minutes at 37°C.
- The tagmentation reaction can then be stopped on ice with addition of a buffer containing SDS (0.1%).

ATAC-seq experiments:

- After cell lysis and nuclei isolation, the nuclei pellets can be incubated with the following mix for 1 tagmentation reaction:

Tagmentation Buffer (2x)	25 µl
Tagmentase loaded	2.5 µl
Digitonin 1%	0.5 µl
Tween20 10%	0.5 µl
PBS	16.5 µl
Nuclease-free water	5 µl
Nuclei pellet*	

** The number of nuclei per reaction will depend on the ATAC-seq experimental design. Successful tagmentation with the proposed protocol has been performed on 50,000 nuclei per reaction.*

- The reaction is then incubated 30 minutes at 37°C.
- The tagmentation reaction can then be stopped by addition of 250 µl of DNA Binding buffer from Diagenode MicroChIP DiaPure Columns (Cat. No. C03040001).
- The tagmented libraries can then be purified using the MicroChIP DiaPure Columns (Cat. No. C03040001), and amplified.

VALIDATION DATA

Diagenode Tagmentase lot 4/aD has been successfully used in various assays as shown in figures 2 and 3.

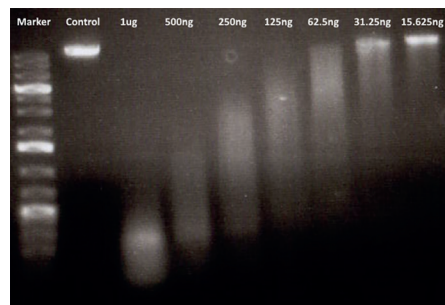


Figure 2. Fragmentation assay on 100 ng of lambda DNA with a wide range of Tagmentase – loaded amounts.

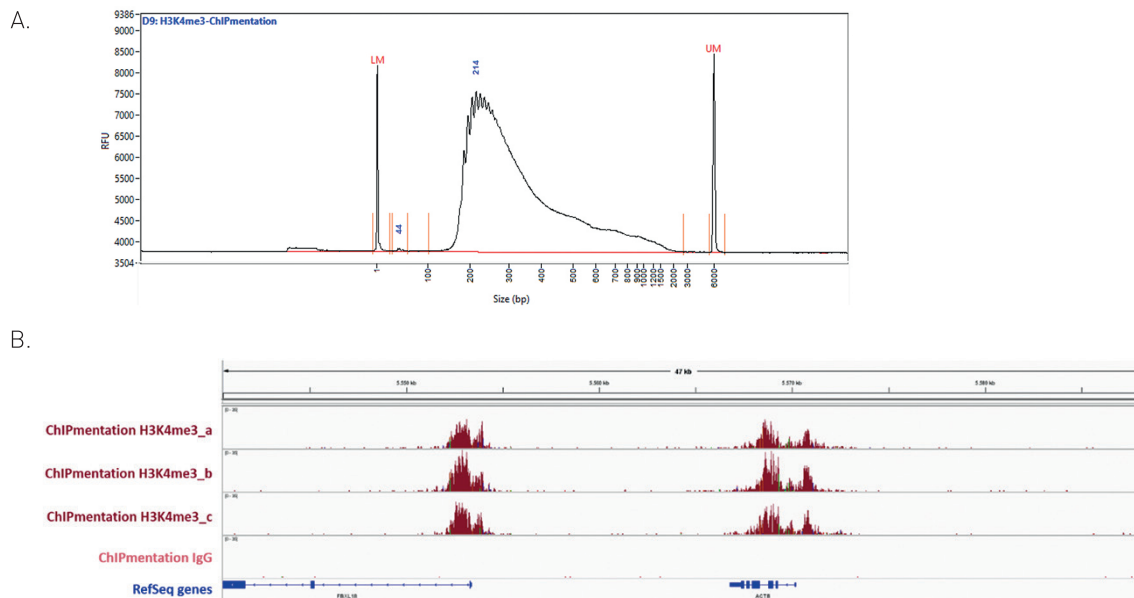


Figure 3. ChIPmentation targeting H3K4me3 on 1M K562 cells, using Diagenode ChIPmentation kit for histones (Cat. No. C01011010) and Tagmentase – loaded. A) Fragment Analyzer profile of one library. B) Sequencing profile of triplicates H3K4me3 libraries and of one IgG library on a representative region.

Reference:

Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. Genome Res. 2014 Dec;24(12):2033-40. doi: 10.1101/gr.177881.114. Epub 2014 Jul 30. PMID: 25079858; PMCID: PMC4248319.