Datasheet for ABIN6952467

Magnetic Concanavalin A Beads (Agarose)

1 Validation | 3 Images | 1 Publication

Go to Product page

Overview

Quantity:	250 µL
Application:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag), Separation (Sep)

Product Details

Purpose:	Immobilization of whole cells through Concanavlin A binding of membrane glyocproteins.
Characteristics:	• Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays are based on a ferrimagnetic core surrounded by an agarose matrix covalently bound via polyurethane links. • In contrast to silica based beads containing superparamagnetic magnetite nanaoparticles, the hydrophilic surface of our Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays reduces the risk of unspecific binding of contaminants. No residual charges are present after conjugation. This minimizes non-specific binding to the matrix. • The weak magnetic moment does not interfere with their solubility in the absence of an external magnet field. Upon exposure to a magnetic field however, the beads show a stronger magnetic reaction than the superparamagnetic beads. They are therefore easier to pull out of a solution using a magnetic separator. • The beads' diameter is with 30 µm considerably larger than for silica based ConA beads. Their capacity is comparable because of the open structure of the agarose carbohydrate network.
Components:	10% suspension of Concanavalin A coated agarose particles with a ferrimagnetic core
Bead Ligand:	Concanavalin A
Bead Matrix:	Magnetic Agarose beads
Bead Size:	30 µm

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Metal ions (calcium and manganese) mediate the binding to Concanavalin A and stabilize is conformation. • The use of buffers with EDTA or other metal chelators must be avoided as it will result in a loss of carbohydrate binding ability.
Protocol:	• Collect approximately 250,000 cells for each sample. • Wash cells 3 times in 1 mL Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl). • Take cells up in a volume of Wash Buffer corresponding to 250,000 cells/mL. • Homogenize Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays slurry by shaking. • Take 10 µL bead slurry for each sample. • Wash beads 3 times with 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$. • Resuspend beads in a volume of Binding Buffer corresponding to the initial volume of bead slurry. • Add beads in Binding Buffer to the cells in Wash Buffer. • Incubate for 30 min at RT to bind cells to Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	20 mM Sodium Acetate pH 6.6, 20% Ethanol
Handling Advice:	Do not freeze the Magnetic ConA Beads (Agarose) for CUT&RUN/CUT&Tag Assays! Vortex bead suspension well before use.
Storage:	4 °C
Expiry Date:	12 months

Publications

Product cited in:	Zambanini, Nordin, Jonasson, Pagella, Cantù: "A new cut&run low volume-urea (LoV-U) protocol optimized for transcriptional co-factors uncovers Wnt/b-catenin tissue-specific genomic targets." in: **Development (Cambridge, England)**, (2022) (PubMed).

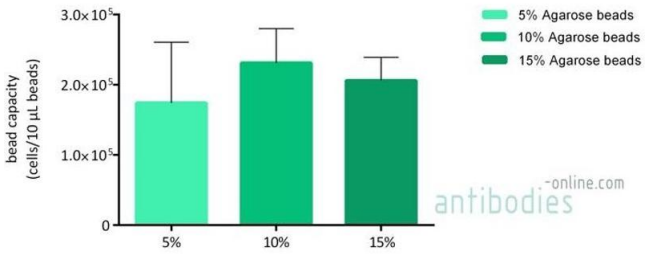
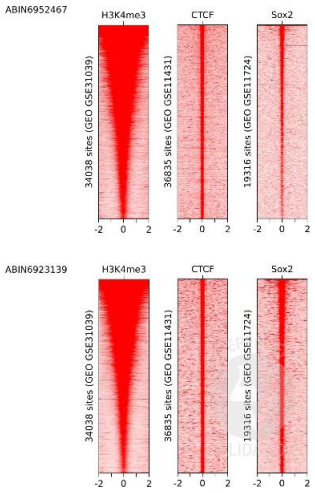
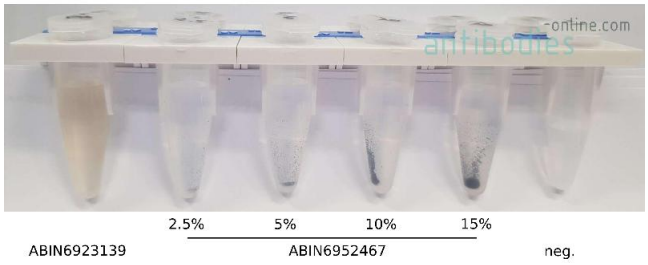


Image 1. Comparison of the number of K562 cells bound to 10 µL Magnetic ConA Beads (Agarose) slurry at different concentrations. ABIN6952467 is provided as a slurry containing 10% Magnetic ConA Beads (Agarose).



Cleavage Under Targets and Release Using Nuclease

Image 2. CUT&RUN data from three experiments using 100,000 mouse ES cells immobilized on 10 µL Magnetic ConA Beads (Agarose) ABIN6952467 (top) or CUT&RUN Concanavalin A Beads ABIN6923139 (bottom) using an H3K4me3 antibody, a CTCF antibody, or a Sox2 antibody. Heat maps show CUT&RUN signal for the three antigens +/- 2kb centered around known sites previously identified by ChIP seq (H3K4me3: GEO GSE31039, 34038 sites; CTCF: GEO GSE11431, 36835 sites; Sox2: GEO GSE11724, 19316 sites). Sites are sorted based on ChIPseq peak intensity from high to low. Images provided by Sarah Hainer, Department of Biological Sciences, University of Pittsburgh



Cleavage Under Targets and Release Using Nuclease

Image 3. 10 µL Magnetic ConA Beads (Agarose) ABIN6952467 at the indicated concentrations were loaded with 2x10^5 K562 cells as described in the protocol section and placed on a magnet stand. 10 µL CUT&RUN Concanavalin A Beads ABIN6923139 (left) and a negative control without beads served as reference.

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Max Planck Institut für Immunbiologie und Epigenetik

Report Number: 104288

Date: Aug 24 2020

Target:	ConA
Lot Number:	cab0304001
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Anti-H3K4me3 antibody
Negative Control:	guinea pig anti-rabbit antibody ABIN101961
Notes:	Passed. ABIN6952467 allows immobilization of cells for CUT&RUN.
Protocol:	• Cell harvest ◦ Harvest 5,000 murine LSK cells per sample to be used at RT. ◦ Centrifuge cell solution 3 min at 600 x g at RT. ◦ Remove the liquid carefully. ◦ Gently resuspend cells in 1 mL Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) by pipetting and transfer cell solution to a 2 mL microcentrifuge tube. ◦ Centrifuge cell solution 3 min at 600 x g at RT and discard the supernatant. ◦ Repeat twice for a total of three washes. ◦ Resuspend cell pellet in 1 mL Wash Buffer by gently pipetting. • Concanavalin A beads preparation ◦ Prepare one 1.5 mL microcentrifuge tube. ◦ Gently resuspend the magnetic silica-based magnetic ConA beads ABIN6923139 or agarose-based magnetic ConA beads ABIN6952467. ◦ Pipette 10 µL Con A Beads slurry for each sample into the 1.5 mL microcentrifuge tube. ◦ Place the tube on a magnet stand until the fluid is clear. Remove the liquid carefully. ◦ Remove the microcentrifuge tube from the magnetic stand. ◦ Pipette 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$) into each tube and resuspend ConA beads by gentle pipetting. ◦ Spin down the liquid from the lid with a quick pulse in a table-top centrifuge. ◦ Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully. ◦ Remove the microcentrifuge tube from the magnetic stand. ◦ Repeat twice for a total of three washes. ◦ Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 10 µL per sample.

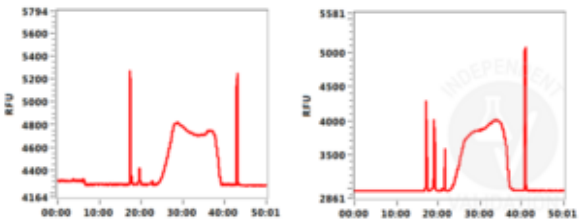
- Cell immobilization – binding to Concanavalin A beads
  - Carefully vortex the cell suspension and add 10 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly and rotate for 10 min at RT.
- Cell permeabilization and antibody binding
  - Divide cell suspension into separate 2 mL microcentrifuge tubes, one for each antibody (5,000 cells per sample).
  - Place the microcentrifuge tubes on a magnetic stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 100 µL Digitonin Wash buffer (wash buffer with 0.025% (wt/vol) Digitonin) supplemented with 2 mM EDTA.
  - Gently vortex the microcentrifuge tubes until the beads are resuspended.
  - For the positive control, add 1 µL anti-H3K4me3 antibody to the respective tube, corresponding to a 1:100 dilution.
  - For the negative control, add 1 µL guinea pig anti rabbit negative control antibody (antibodies-online, ABIN101961, lot NE-200-12190001) to the respective tube, corresponding to a 1:100 dilution.
  - Rotate the microcentrifuge tubes ON at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- pA-MNase Binding
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Vortex the sample at low speed and add 150 µL pA-MNase solution at 700 ng/mL per sample, gently resuspending the beads by pipetting.
  - Rotate the microcentrifuge tubes for 1 h at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 mL pipette tip.
  - Repeat once for a total of two washes.
- MNase digestion and release of pA-MNase-antibody-chromatin complexes
  - Spin down the liquid from the lid with a quick pulse in a table-top centrifuge.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 100 µL Digitonin Wash buffer per sample along the side of the tube.

- Place tubes in a heat block, kept on ice, and allow to chill.
- Add 2 µL 0.1 M $CaCl_2$ to each sample.
- Incubate tubes at 0 °C for 30 min.
- Add 100 µL 2xSTOP buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.05% (wt/vol) Digitonin, 100 µg/mL RNAse A, 50 µg/mL Glycogen).
- Incubate tubes at 37 °C for 30 min.
- Place the tubes on a magnet stand until the fluid is clear.
- Transfer the supernatant containing the pA-MNase-bound digested chromatin fragments to fresh 1.5 mL microcentrifuge tubes.

- DNA extraction
  - Add 2 µL 10% SDS to a final concentration of 0.1% and 2.5 µL Proteinase K (20 mg/mL) to each supernatant.
  - Gently vortex tubes at a low speed of approximately 1,100 rpm.
  - Incubate tubes at 50 °C for 1 h.
  - Add 200 µL PCI to tube.
  - Vortex tubes thoroughly at high speed until the liquid appears milky.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at RT for 5 min.
  - Carefully transfer the upper aqueous phase to a fresh 1.5 mL microcentrifuge tube containing 200 µL chloroform:isoamyl alcohol 24:1.
  - Vortex tubes thoroughly at high speed until the liquid appears milky.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 5 min.
  - Carefully transfer to upper aqueous phase to a fresh 1.5 mL microcentrifuge tube containing 2 µL glycogen (diluted 1:10 to 2 mg/mL from the 20 mg/mL stock solution).
  - Add 20 µL 3 M NaOAc pH 5.2.
  - Add 400 µL 100% ethanol.
  - Place tubes for at -20 °C ON.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 5min.
  - Remove the liquid carefully with a pipette.
  - Wash pellet with 1ml 70% ethanol.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 1 min.
  - Remove the liquid carefully with a pipette.
  - Air-dry the pellet, then dissolve in 30 µL 1 mM Tris-HCl, 0.1 mM EDTA.
- Library preparation and sequencing
- Read mapping and Peak calling

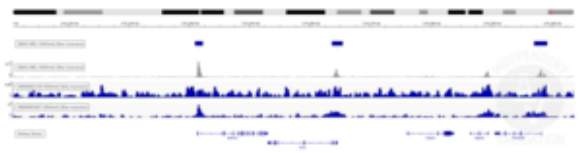
Experimental Notes:

- Transcription start sites were identified through CUT&RUN on LSK cells immobilized on magnetic ConA beads ABIN6952467 using an H3K4me3 antibody. Identified peaks were consistent with the Histone Mods by ChIP-seq from ENCODE (NCBI37/mm9).
- Background signal using the agarose based ConA beads ABIN6952467 appeared to be lower than in a parallel experiment using silica based ConA beads for cell immobilization.



Validation image no. 1 for Magnetic Concanavalin A Beads (Agarose) (ABIN6952467)

Fragment Analyzer profiles comparing fragment size distributions between reads obtained from CUT&RUN using an anti-H3K4me3 CUT&RUN Positive Control antibody in conjunction with silica-based magnetic ConA beads ABIN6923139 (left) or agarose-based magnetic ConA beads ABIN6952467 (right)



Validation image no. 2 for Magnetic Concanavalin A Beads (Agarose) (ABIN6952467)

Alignment to mouse NCBI37/mm9 reference genome of reads from CUT&RUN targeting H3K4me3 in LSK cells using ABIN6952467 (tracks 3 and 4) and ENCODE H3K4me3 ChIP-seq signal (accession ENCFF001MZZ, track 2; peaks in track 1).