Datasheet for ABIN6952467

**Magnetic Concanavalin A Beads (Agarose)**

### 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 Concanavalin A binding of membrane glycoproteins. |
| 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 nanoparticles, 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

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<th>Application Notes:</th>
<th>Optimal working dilution should be determined by the investigator.</th>
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| Comment:           | • Metal ions (calcium and manganese) mediate the binding to Concanavalin A and stabilize its 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

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| 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

**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).

**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.

**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.
**Validation report #104288 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)**

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

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**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.

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**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 CaCl², 1 mM MnCl²) 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.
Validation report #104288 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

- 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.
Validation report #104288 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

- 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 5 min.
    - 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**

**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 ENCF001MZZ, track 2; peaks in track 1).