AHARIBO for *de novo* proteome analysis

Product	Catalog no.	Rxns
AHARIBO protein	#AHA003-P	6

Shipping: dry ice and +4°C

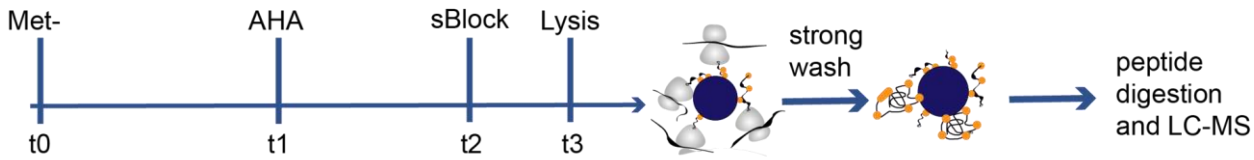
Storage Conditions: store components as indicated on data sheet

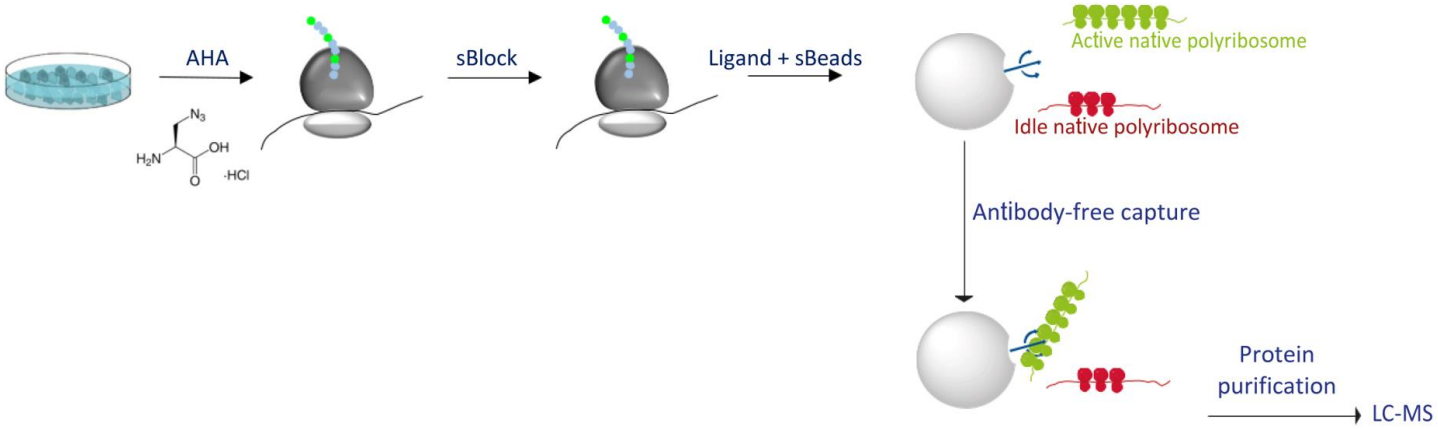
Shelf Life: 12 months

Description:

AHARIBO for *de novo* proteome analysis is a product based on an IMMAGINA proprietary technology called Minimally Invasive Non-canonical Amino acid Tagging and Isolation of Ribosomes (RiboMINATI). RiboMINATI is designed for the isolation of active polyribosomes, associated RNAs and nascent peptides. The protocol is based on the pulse incubation of cell cultures with azidohomoalanine (AHA). AHA-treated cells are incubated with a proprietary small molecule (sBlock) that blocks nascent peptides on the translating ribosomes, and lysed. The newly synthesized AHA-labeled peptides are then used as tags for the separation of active ribosome complexes through chemical interactions with proprietary smart beads. Finally, nascent peptides associated with ribosome complexes are isolated and purified for downstream analyses.

Principle and Procedure





L-azidohomoalanine (AHA) provides a fast, sensitive, non-toxic and non-radioactive labeling.
AHA is an amino acid analog bearing a very small modification consisting of an azide moiety that can be safely fed to cultured cells and incorporated into proteins during active protein synthesis.

Reagents provided

Product (label)	Catalog no.	Storage Conditions	Quantity
AHARIBO for *de novo* proteome analysis	#AHA003-P	according to user's manual	6 reactions
W-buffer (WB)	#RM2	4°C	15 mL
dBeads (dB)	#RM10	4°C	600 µL
Urea Washing Solution (UWS)	#RM11	4°C	15 mL
AHA (100 mM)	#RM5	-20°C	60 µL
Lysis buffer (LB)	#RM6	-20°C	0.5 mL
L-Leucine (80 mM, LL)	#RM7	-20°C	200 µL
sBlock (1000x)	#RM8	-20°C	50 µL

AHARIBO includes vials with (i) the blocker molecule (sBlock), (ii) smart beads (dBeads), (iii) AHA; and tubes with buffers for (i) lysis, (ii) bead functionalization and pulldown. The kit contains reagents for 6 samples processed from a 6-well dish.

Shelf life: 12 months from the delivery date

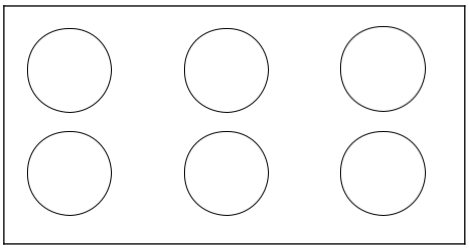
Reagents and equipment to be supplied by the user

- o - RiboLock RNase inhibitor (Thermo Scientific catalog no. EO0381)
- o - RNase free water / DEPC water Dnase I (Thermo Scientific catalog no. EN0521)
- o - Dnase I (Thermo Scientific catalog no. EN0521)
- o - Methionine-free medium (Thermo Scientific catalog no. 300330)
- o - Protease inhibitor cocktail (Cell Signaling catalog no. 5871S)
- o - Fetal Bovine Serum (Thermo Scientific catalog no. A3840001)
- o - Sodium deoxycholate 10% in nuclease-free water

- o - Nanodrop ND-1000 UV-VIS Spectrophotometer
- o - microcentrifuge and nonstick RNase-free microcentrifuge tubes (0.2 mL and 1.5 mL)
- o - Automatic wheel (rotator)
- o - Magnetic separation device for 1.5 mL tubes
- o - Mixer

Work always in an RNase-free environment

Reagents are optimized for experiments in 6-well plates



Cell Seeding

We recommend using cells at 70 to 90% confluence. Typically, for experiments in 6-well plates, 150000-250000 adherent cells are seeded per well in 2 ml of cell growth medium.

Before starting the experiment

Preparation of the lysis buffer: Keep the required optimal volume of lysis buffer on ice and add the following components: Sodium deoxycholate (1% final concentration), 5U/mL DNase I, sBlock, 1x proteinase inhibitor and 200 U/mL RiboLock RNase inhibitor

Example for a 1 well of a 6 well plate						
Optimal final volume	Lysis buffer	SDC (10%)	DNase I	RiboLock	sBlock	P.inhibitor
50 µL	44 µL	5 µL	0.25 U	10 U	0.5 µL	1x

Preparation of the methionine-free medium: Add 0.1 mL of FBS, antibiotics if required and 10 µL of L-leucine to 0.89 mL of Methionine-free medium (Thermo Scientific catalog no. 30030)

Beads washing (1 reaction)

- Remove dBeads from 4°C and place the tube at RT
- Vortex dBeads for >30 sec

! Do not let the beads dry out at any point

- Transfer 100 µL of dBeads to a new 1.5 mL tube
- Place the tube on a magnetic separation device to separate the beads. Remove supernatant
- Wash the beads twice in a volume of 300 µL of W-buffer
- Place the tube onto the magnetic rack and discard the supernatant. Immediately add 50 µL of W-buffer supplemented with RiboLock (diluted 1 : 2000) to the beads.
- Resuspend the beads
- Keep on ice until later use

Step 1. Cell treatment and cell lysis - sample prep for 1 well

(optional) To enhance the incorporation of AHA, the growing cell medium can be replaced with 2 mL/well of DMEM medium (with L-glutamine, Sodium Pyruvate and Glucose) supplemented with 0.5% FBS (37°C) for 8 hours

- Wash cells (80% confluent) once with PBS, add 1 mL/well of previously prepared methionine-free medium to the cells and incubate the dish at 37°C for 40 minutes to deplete methionine reserves
- Add 10 µL of AHA reagent to the cells gently mix and incubate for 5 min at 37°C
- Add 2.6 µL of sBlock to the cells gently mix and incubate for 10 min at 37°C
- Place the plate on ice and wash cells twice with 1000 µL/well of cold PBS containing sBlock (diluted 1:400)
- Remove residual PBS with a pipette
- Add 40 µL of lysis buffer (prepared before) to the well and lyse cells using a scraper
- Collect the cell lysate in a 1.5 mL microcentrifuge tube and pellet the nuclei / cell debris by centrifugation at 20,000 x g for 5 min at 4°C

- Transfer the supernatant to a new tube and keep it on ice for 10 min. With Nanodrop, check the absorbance of the cell lysate at 260 nm with lysis buffer as blank subtraction (using the “nucleic acid” function of the Nanodrop)

Step 2. Click reaction – 1 reaction

- Start with a total volume of lysate corresponding to 0.2 a.u (260 nm). You may have to scale the lysate input amount up or down depending on the specific biological model. Optimal results will be obtained when a good trade-off between signal intensity and background noise is reached
- Supplement 0.5 mL of W-buffer with 0.25 µL of RiboLock to use in the next step
- Add the previously prepared W-buffer to the cell lysate to a final total volume of 100 µL
- Add 50 µL of dBeads (prepared before) to each sample
- Incubate for 60 min, on a wheel in slow motion (9 rpm) at 4°C
- Take the tubes off the wheel
- DO NOT CENTRIFUGATE. Place the tubes on a magnetic rack on ice and remove supernatant
- Wash the beads with 500 µL of UWS incubating for 10 minutes on a thermomixer at 1000 rpm at room temperature
- Repeat the washing step 4 more times
- After the final wash, remove the supernatant and resuspend the beads in 200 µL of water
- Polypeptides are bound to the beads. Transfer the suspension (beads in water) to a new 1.5 mL tube

At this point, samples are ready for reduction, alkylation and protease digestion in preparation for proteomic analysis. Perform the digestion on beads. IMPORTANT!! UWS solution contains CHAPS. Samples may therefore carry residual CHAPS.