

AHARIBO RNA and Protein System

Product	Catalog no	Rxns.
AHARIBO RNA and Protein System	#AHA-R6P6	12

Shipping: Blue Ice

<u>Storage Conditions</u>: store components according to the storage conditions reported on the labels, and on Page 6 of this manual.

Shelf Life: 12 months

<u>Description</u>: AHARIBO is a sample preparation kit for selective, parallel isolation of active ribosomes, full-length translated RNAs and corresponding *de novo* synthesized peptides. AHARIBO represents an effective tool to explore quantitative relationship between transcripts and protein levels. The protocol is based on a pulse incubation of cells with I-azidohomoalanine (AHA) followed by sBlock treatment to stabilize nascent peptides on the translating ribosomes. The newly synthesized AHA-labelled peptides are then used as tags for the separation of active ribosome complexes through chemical interactions with magnetic beads.

AHARIBO RNA and Protein System enables to perform RNA analysis such as qPCR or RNAseq on translating RNAs in parallel to de novo protein synthesis analysis.

Suitable for: Eukaryotic cell lines

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 1 of pag 20

Table of Contents

Kit storage info	3
Additionally Required Materials	3
INTRODUCTION	4
WORKFLOW OVERVIEW	4
A. SAMPLE PREPARATION	5
A.1 Sample Recommendations	5
A.1.1. Cell Medium Recommendations	5
A.2 AU calculation - Input lysate quantification	5
A.2.1 Measure Lysate AU	5
A2.2 Calculate the volume of lysate and diluting buffer needed for the pulldown	6
Optimal Workflow Recommendations	7
B. Pull-down of active ribosomes and full-length translated RNAs	8
Step B1. CELL LYSIS	9
Before starting the experiment – Supplemented Lysis Buffer (SLB)	9
Adherent Cells lysis	9
Suspension Cells lysis	10
Step B2. BEADS FUNCTIONALIZATION	12
Step B3. Click reaction (material for 1 reaction)	14
STEP 4. RNA extraction	15
APPENDIX	18
App.1 Lysis buffer supplementation issues	18
App.2 Check proper beads functionalization (for Step B2 – Beads functionalization)	19

Kit storage info

	Qty.	Storage
4°C components	1 box	4°C
-20°C components	1 box	-20°C

 Table 1. Kit composition (in boxes) and storage temperature.

Additionally Required Materials

- RiboLock RNase inhibitor (Thermo Scientific catalog no. EO0381)
- o RNase free water / DEPC water
- Dnase I (Thermo Scientific catalog no. EN0521)
- Methionine-free medium (e.g., for DMEM Thermo Scientific catalog no. 30030)
- Sodium deoxycholate 10% in nuclease-free water
- Nanodrop ND-1000 UV-VIS Spectrophotometer
- o microcentrifuge and nonstick RNase-free microcentrifuge tubes (1.5 mL)
- Automatic wheel (rotator)
- Magnetic separation device for 1.5 mL tubes
- o Mixer
- o Vortex
- o DMSO
- o PBS, Phosphate Buffered Saline, 1X Solution, pH 7.4
- Glyco Blue (Thermo Scientific catalog no. AM9516)
- Isopropanol (Sigma catalog no. 278475)
- Proteinase K (Qiagen catalog no. 19131)
- SDS 10% in nuclease-free water
- o 70% ethanol
- Acid Phenol:chloroform:isoamyl alcohol (Ambion catalog no. AM9720)
- Any commercially available kit for DNAse treatment and RNA cleanup

Mod 080262 v1 Date Exp/rev 24/01/2024 Edited by MA PB Pag 3 d	of pag 20
---	-----------

INTRODUCTION

AHARIBO (AHA-mediated RIBOsome isolation) is an innovative solution for proteogenomic analysis. AHARIBO represents an effective tool to explore quantitative relationships between transcript and protein levels, offering a reliable and accurate approach for capturing active translation processes.

AHARIBO was developed to overcome the limits of classical methods such as polysome profiling or affinity purification-based techniques that are characterized by labor-intensive protocols and relatively poor correlations between mRNA and protein levels.

The AHARIBO RNA and Protein System (#AHA-R6P6) allows the parallel isolation and downstream analysis of translated RNAs (by RNAseq or qPCR) and the associated newly synthesized proteins



WORKFLOW OVERVIEW

Fig.1 Overview of the AHARIBO RNA and Protein System workflow. The cell manipulation is depicted in the workflow with the different components needed added during different time points (t0-t3) before pulldown of the nascent peptide with the beads and subsequent RNA extraction (top) or protein extraction (bottom).

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 4 of pag 20

A. SAMPLE PREPARATION

A.1 Sample Recommendations

Reagents are optimized for experiments in 6-well plates, for different quantities, please scale up or down the amount of lysis buffer utilized in Step B1.

Typically, for experiments in 6-well plates, 150000-250000 adherent cells are seeded per well in 2 ml of cell growth medium. We recommend treating cells with AHA at 70 to 90% confluence when the cells are actively dividing. Please note that the amount of newly synthetize proteins with the translational machinery that can be isolated from a sample is strongly affected by its translational state and must be considered when programming experiments with the IMMAGINA AHARIBO RNA System. For instance, two lysates similarly concentrated (i.e., similar Abs260nm) but from different cell types or specimens (e.g. human vs mouse, or immortalized vs primary), or with different treatments (e.g. drugs and transfection reagents) could have completely different amounts of translating ribosomes, leading to opposite outcomes.

A.1.1. Cell Medium Recommendations

To prepare Methionine free complete medium, you should supplement the specific medium for your cell lines with all the components needed (e.g., Penicillin, L-glutamine, FBS, growth factors, etc.). If the Methionine-free medium that you purchase is also depleted from L-leucine, please add 10 μ L/mL of L-leucine (80 mM ready-to-use provided in the kit #IBT0441).

The complete Methionine-free medium must be prewarmed to 37°C or the right growing temperature before addition to the cells, to allow proper growth.

A.2 AU calculation - Input lysate quantification

A.2.1 Measure Lysate AU

Cells should be lysed following Step B1 a, or b instructions depending on your specimen type. The AU of your sample is measured using a spectrophotometer, most commonly a Nanodrop. Set the instrument so to measure the Abs at 260 nm (usually Nucleic Acid function) and measure the absorbance of your lysate using the Supplemented Lysis Buffer (SLB) as blank (see Before starting the experiment – Lysis Buffer Supplementing & Table 3). The use of different lysis buffers are strongly discouraged because it may interfere with the efficiency of ribosome pull-down and with the AU calculation (some components may absorb at 260 nm).

<u>If the instrument does not allow to use of the SLB as blank</u>, please use water instead, then record the absorbance of both the SLB and the lysate and subtract the absorbance of the SLB to the lysate.

Example:

- \Box Supplemented Lysis buffer SLB Abs260nm = 7 AU
- \Box Specimen Abs260nm = 17 AU
- □ Absorbance value of lysate = 17 7 = 10 AU

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 5 of pag 20

A2.2 Calculate the volume of lysate and diluting buffer needed for the pulldown

The absorbance of your sample depends on your sample characteristics (type of cell and amount) if resuspended in the same volume of lysis buffer.

In Step X, to calculate the volume of lysate to dilute in supplemented WB buffer (SWB) to reach 2 AU in 100 µL, follow the examples below.

Example 1: Nanodrop absorbance value of lysate at 260 nm = 10 AU.

- \Box 2 AU (final quantity) * 100 μ L (final volume) = 200 AU* μ L
- \Box 200 AU*µL /10 AU (lysate absorbance) = 20 µL of lysate to pipette
- $\Box~$ Put 20 μL of lysate in 80 μL of SWB buffer.

Example 2: Nanodrop absorbance value of lysate at 260 nm = 4 AU.

- $\Box~$ 2 AU (final quantity) * 100 μL (final volume) = 200 AU* μL
- \Box 200 AU*µL /4 AU (lysate absorbance) = 50 µL of lysate to pipette
- $\hfill\square$ Put 50 μL of lysate in 50 μL of SWB buffer.

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 6 of pag 20

Optimal Workflow Recommendations

- Please run up to 6 samples in parallel. Longer manipulation time may introduce an unwanted variability between the first and the last sample.
- Allocate at least 1 day for the completion of the entire workflow.
- This protocol has been optimized to perform all the reactions from cells growing at 37°C in a 6 well plate format. For other format and temperatures needed for your cell line, please adjust lysis buffer volume and temperature accordingly.
- The pulldown has been optimized for starting with 2 AU (Abs260nm) in 100 μL of diluted lysate.

Mod 080262 v1 Date Exp/rev 24/01/2024 Edited by MA PB Pag 7 of p	Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 7 of pag 2
--	------------	----	-------------------------	-----------------	--	----------------

B. Pull-down of active ribosomes and full-length translated RNAs

AHARIBO RNA System components and additional required materials needed in this section:

Step N	Kit component	Cat. nr.	Volume	Storage	Туре		Vial cap color
B1	Lysis buffer (LB)	# IBT0034	0.6 mL	-20°C	Vial		clear
B1	SDC 10%	Additionally Required Material					
B1	DNAse I	Additionally Required Material					
B1	RiboLock RNase Inhibitor	Additionally Required Material					
B1	sBlock	# IBT0451	40 µL	-20°C	Vial	100 A	clear
B1	PBS	Additionally Required Material					
B1	L-azidohomoalanine (100 mM) (AHA)	# IBT0431	120 µL	-20°C	Vial	100 A	clear
B1	L-Leucine (80mM) (LL)	# IBT0441	120 µL	-20°C	Vial		clear
B2	Ligand (5mM) (G)	# IBT0471	30 µL	-20°C	Vial	4 1 4 4 4 4 4 4	clear
B2,3	Wash Buffer (WB)	# IBT0072	15 mL	4°C	Bottle		
B2,3	Washing Solution (WSS)	# IBT0461	15 mL	4°C	Bottle		
B2	sBeads	# IBT0043	320 µL	4°C	Vial		Blue
B2	DMSO	Additionally Required Material					
B4	Proteinase K	Additionally Required Material					
B4	SDS 10%	Additionally Required Material					
B4	Acid Phenol: Chloroform:Isoamyl Alcohol	Additionally Required Material					
B4	Isopropanol	Additionally Required Material					
B4	GlycoBlue	Additionally Required Material					
B4	70% cold ethanol	Additionally Required Material					

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 8 of pag 20
			· · · · · · · · · · · · · · · · · · ·	.0

Step B1. CELL LYSIS

Before starting the experiment – Supplemented Lysis Buffer (SLB)

To ensure optimal reproducibility we recommend producing a fresh Supplemented Lysis Buffer (SLB) aliquot for each new experiment, right before proceeding with the Lysis Step. Combine the SLB by following Table 2 instructions and multiply the volumes according to the number of samples being processed (N). Please combine the different reagents following the left-to-right order. Note that the volumes for the protocol are of supplemented lysis buffer suggested for a well of a 6 wells plate is 50 μ L. For other sizes of dishes, please use proportional volumes.

	Lysis buffer (LB)	Sodium deoxycholate (SDC) 10% (W/V)	DNase I 1 U/µL	RiboLock RNase Inhibitor 40 U/µL	sBlock	Final Volume
N=1	43.5 μL	5 µL	0.25 μL	0.25 µL	0.5 µL	50 µL
N=						

 Table 2. Recipe for the supplementation of the provided lysis buffer.

The SLB final concentration is Sodium deoxycholate (1%), DNase I (5U/mL), and RiboLock RNase Inhibitor (200 U/mL).

Please if the SLB appears as a whiteish and cloudy solution, do not proceed and check Appendix 1.

Adherent Cells lysis

- □ B1.1a Remove the medium from the 6 well plate.
- B1.2a Wash cells (80% confluency) by adding 1 mL of PBS and removing it after the wash.
 Repeat the wash.
- □ B1.3a Add 1 mL/well of methionine-free medium (supplemented as needed) to the cells and incubate at 37°C for 40 minutes.
- \square B1.4a Add 10 µL of AHA reagent to the cells, and incubate for 5 min at 37°C.
- \square B1.5a Add 2.6 µL sBlock to the cells, and incubate for 5 min at 37°C.
- □ B1.6a Place the plate on ice and wash the cells with 1mL/well of cold PBS.
- □ B1.7a Remove all residual PBS with a pipette. All the PBS must be removed before proceeding with the lysis to avoid diluting the lysis buffer.
- □ **B1.8a** Perform the lysis directly adding 45 µL of the complete **Supplemented Lysis Buffer** (Table 2) to each cell well and scrape vigorously. Mechanical scraping helps the downstream

Mod 080262 v1 Date Exp/rev 24/01/2024 Edited by MA PB Pag 9 of pag	Mod 080262 v1	1 Date Exp/rev 24/01	1/2024 Edited by MA PB		Pag 9 of pag 20
--	---------------	----------------------	------------------------	--	-----------------

processing by disrupting the cell membrane and releasing the cellular contents, including ribosomes. To ensure good lysis, follow these guidelines for mechanical scraping:

- Before scraping, make sure you are working in a sterile environment using appropriate aseptic techniques.
- Prepare your sample by adding the necessary lysis buffer or solution as per the protocol.
- Using a suitable tool such as a cell scraper, spatula, or pipette tip, gently scrape the surface of the cell culture dish or tissue to dislodge the cells.
- Apply consistent but gentle pressure to ensure thorough scraping while avoiding excessive force that may introduce debris.
- Scrape in a systematic manner, covering the entire surface area to ensure an even distribution of lysed cells.
- Continue scraping until you observe the desired level of cell detachment and release of cellular material.
- Transfer the lysate to a suitable collection vessel, such as a microcentrifuge tube, for further processing or analysis.
- □ **B1.9a** Collect the cell lysate in a 1.5 mL microcentrifuge tube and pellet the cell debris and nuclei by centrifugation at 20,000 g for 5 min at 4°C.
- □ **B1.10**a Transfer the supernatant to a new tube and keep it on ice for 20 min.
- □ B1.11a Check the absorbance of the cell lysate at 260 nm, we suggest using a Nanodrop setting the "nucleic acid" function and using 1.5 µL of the supplemented lysis buffer as blank (for troubleshooting check A.3 AU calculation Input lysate quantification). If the sample is not processed the same day, please store the sample at -80°C or in a cryogenic storage system to maintain its stability until further processing.

Suspension Cells lysis

- □ **B1.1a** Collect the suspension cells (80% confluency) by pelleting them using a centrifuge (e.g., centrifugate at 300 g for 5 min at RT). Remove the supernatant.
- □ **B1.2a** Wash pelleted cells with 1 mL of PBS. Pellet them again by centrifugation and remove the supernatant. Repeat the wash and remove completely the supernatant after centrifugation.
- □ B1.3a Add 1 mL/well of methionine-free medium (supplemented as needed) to the cells and incubate at 37°C for 40 minutes.
- \square B1.4a Add 10 µL of AHA reagent to the cells, and incubate for 5 min at 37°C.
- \square B1.5a Add 2.6 µL sBlock to the cells, and incubate for 5 min at 37°C.
- B1.6a Collect the treated suspension cells by pelleting. Wash the cells with 1mL of cold PBS.
 Pellet them again by centrifugation.
- □ B1.7a Remove all residual PBS with a pipette. All the PBS must be removed before proceeding with the lysis to avoid diluting the lysis buffer.

Mod 080262 v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 10 of pag 20
---------------	-------------------------	-----------------	--	------------------

- B1.7a Perform the lysis directly adding 45 µL of the complete Supplemented Lysis Buffer (Table 2) to the cell pellet. Lysate the cells pipetting up and down at least 30 times with a 200 µL pipette without generating bubbles.
- □ **B1.8a** Collect the cell lysate in a 1.5 mL microcentrifuge tube and pellet the cell debris and nuclei by centrifugation at 20,000 g for 5 min at 4°C.
- □ B1.9a Transfer the supernatant to a new tube and keep it on ice for 20 min.
- □ B1.10a Check the absorbance of the cell lysate at 260 nm, we suggest using a Nanodrop setting the "nucleic acid" function and using 1.5 µL of the supplemented lysis buffer as blank (for troubleshooting check A.3 AU calculation Input lysate quantification). If the sample is not processed the same day, please store the sample at -80°C or in a cryogenic storage system to maintain its stability until further processing.

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 11 of pag 20
------------	----	-------------------------	-----------------	--	------------------

Step B2. BEADS FUNCTIONALIZATION

▲ DO NOT LET THE BEADS DRY OUT AT ANY POINT!

Beads functionalization steps:

The amount of beads that need to be functionalized per experiment depends on the number of samples. For clarity, the following steps refer to one reaction. For multiple samples, it is possible to functionalize beads for more than one reaction in one single tube (within its capacity).

- □ **B2.1** Remove the **sBeads** from 4°C and place the tube at RT for at least 15 min.
- B2.2 Prepare the Ligand Solution by adding 2 μL of Ligand to 50 μL of WSS buffer and mix well by vortexing.
- \square B2.3 Keep at least 2 µL of the Ligand Solution for security checkpoint (see grey box below).
- \square **B2.4** Vortex the sBeads tube thoroughly for > 30 sec.
- □ B2.5 Put 50 µL of sBeads in a new 1.5 mL tube. Place the tube on a magnet to separate the sBeads. Visually inspect that all the beads are attached to the magnet and remove the supernatant.
- B2.6 Remove the tube from the magnet and wash the sBeads with 200 μL of WSS buffer for 2 min shaking at 1,200 rpm at RT. Place back the tube and the magnet and remove the supernatant.
- \square B2.7 Resuspend the sbeads with 50 µL of Ligand solution.
- □ B2.8 Incubate for 1h at RT in a shaker at 1,200 rpm. Do not allow beads to sediment.
- B2.9 After the incubation, place the tube on a magnet and store the supernatant (unbound) in a new 1.5 mL eppendorf for the security checkpoint (see below).
- B2.10 Wash the functionalized sBeads two times with 200 μL of WB for 2 min with shaking at 1,200 rpm at RT. After the first wash, put the tube on the magnet to remove the supernatant before adding the solution. After the second wash, place the tube on the magnet and remove the supernatant.
- \square B2.11 Supplement 600 µL of WB with 0.3 µL of RiboLock to create supplemented WB (SWB).
- B2.12 Wash the sBeads with 200 μL of SWB for 2 min with shaking at 1,200 rpm at RT. Put the tube on the magnet to remove the supernatant.
- $\hfill\square$ B2.13 Resuspend the functionalized sbeads with 100 μL of SWB.
- □ B2.14 If the beads were functionalized for more than one reaction, equally divide the functionalized beads in individual tubes according to the (N) number of samples you are processing.

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 12 of pag 20

▲ Security Check Point

You can check for proper bead functionalization by following the instructions in Appendix 2. This step is optional, and it is useful to validate the proper execution of the abovementioned functionalization steps.

	Mod 080262 v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 13 of pag 20
--	---------------	-------------------------	-----------------	--	------------------

Step B3. Click reaction (material for 1 reaction)

- B3.1 Dilute the lysate obtained at the end of STEP B1, with SWB buffer supplemented in Step B2.11 to obtain a final value of 2 AU in 100 µL (see A2.3 Calculate the volume of lysate and diluting buffer needed for the pulldown section for more information).
- \square **B3.2** Add 100 µL of sBeads prepared in **STEP B2**.
- □ **B3.3** Incubate for 60 min on a wheel in slow motion (3-10 rpm) at 4°C.
- B3.4 Remove the tubes from the wheel. DO NOT CENTRIFUGATE but allow the entire solution with the beads to settle at the bottom of the tube. If residual solution is present on the lid, pull down the beads by gently flicking down the tube by hand 2 or 3 times.
- □ **B3.4** Place the tube onto the magnet and let the beads collect for 2 3 minutes or until the supernatant is completely clear. Remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
- **B3.5** Add 700 μL of **WSS** solution to the beads (do not resuspend the beads).
- □ **B3.6** Incubate for 10 minutes on a wheel in slow motion (9 rpm) at 4°C.
- B3.7 Place the tube onto the magnet and let the beads collect for 2 3 minutes or until the supernatant is completely clear. Remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
- □ B3.8 Repeat the steps from B3.5 to B3.7
- \square **B3.9** Resuspend the beads in 200 µL of SWB (Step B2.11).
- □ **B3.10** Transfer the beads suspension to a new nuclease-free 1.5 mL tube. Note that ribosomes and RNA are bound to the beads!

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 14 of pag 20
			,	0 10

STEP B4. RNA extraction

1 is important to use the ACID phenol:chloroform to avoid DNA contamination.

- \square B4.1 Add 20 μL (1% final concentration) of 10 % SDS, 7 μL of Proteinase K and incubate at 37°C in a water bath for 60 minutes.
- □ **B4.2** Add an equal volume of ACID Phenol:Chloroform:Isoamyl alcohol.
- □ **B4.3** Vortex and centrifugate at 14,000 x g for 5 min.
- □ **B4.4** If there is no phase separation, add 20 µL of NaCl 2M in DEPC water and repeat the centrifugation.
- □ **B4.5** Keep the aqueous phase and transfer it into a new vial.
- \square **B4.6** Add 500 µL of isopropanol and 2 µL of GlycoBlue.
- □ **B4.7** Mix and incubate at RT for 3 min, then store at -80°C for at least 2 hours (up to 3 days).
- □ **B4.8** Thaw samples on ice and centrifuge for 30 min at 20,000g at 4°C, then remove supernatant.
- B4.9 Add 500 µL of 70% ice-cold ethanol to the pellet and centrifuge for 10 min at 20,000g at 4°C, then remove the supernatant.
- □ **B4.10** Let the pellet air-dry for 5 min.
- \square **B4.11** Resuspend the pellet in 12 µL of nuclease-free water.
- B4.12 If needed, perform DNAse treatment on your sample and clean up the RNA following the manufacture's instruction of your available kit.
- □ **B4.13** Quantify the RNA with Nanodrop at 260 nm. The RNA is now ready for qPCR, ribodepletion and/or sequencing.

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 15 of pag 20
------------	----	-------------------------	-----------------	--	------------------

C. Pull-down of nascent peptide and active ribosomes

AHARIBO Protein System components and additional required materials needed in this section:

Step N	Kit component	Cat. nr.	Volume	Storage	Туре	Vial cap color
B2	Wash Buffer (WB)	# IBT0072	15 mL	4°C	Bottle	
B2	Urea Washing Solution (UWS)	# IBT0481	18 mL	4°C	Bottle	
B2	dBeads	# IBT0044	600 µL	4°C	Vial	Blue

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 16 of pag 20
11100 000202	• -	Dute Exp/101/2021	Earcea by Mirth B	1 48 10 01 948 20

Step C2. Click reaction (material for 1 reaction)

- □ **C2.1** Remove the **dBeads** from 4°C and place the tube at RT for at least 15 min.
- \Box C2.2 Dilute the lysate obtained at the end of STEP B1, with WB to obtain a final value of 2 AU in 100 µL (see A2.2 Calculate the volume of lysate and diluting buffer needed for the pulldown section for more information).
- \Box C2.3 Add 100 µL of dBeads.
- □ C2.4 Incubate for 60 min on a wheel in slow motion (3-10 rpm) at 4°C.
- C2.5 Remove the tubes from the wheel. DO NOT CENTRIFUGATE but allow the entire solution with the beads to settle at the bottom of the tube. If residual solution is present on the lid, pull down the beads by gently flicking down the tube by hand 2 or 3 times.
- C2.6 Place the tube onto the magnet and let the beads collect for 2 3 minutes or until the supernatant is completely clear. Remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
- \Box C2.7 Add 500 µL of UWS solution to the beads.
- □ **C2.8** Incubate for 5 minutes on a thermomixer at 1,000 rpm at RT.
- □ C2.9 Place the tube onto the magnet and remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
- □ C2.10 Repeat the steps from C2.7 to C2.9
- \Box C2.11 Resuspend the beads in 200 µL of water.
- C2.12 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.

MOG USU262 VI Date Exp/rev 24/01/2024 Edited by MA PB Pag 17 of pag	Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 17 of pag 20
---	------------	----	-------------------------	-----------------	--	------------------

APPENDIX

App.1 Lysis buffer supplementation issues

Please check if, after adding Sodium deoxycholate a whiteish and cloudy solution appears. If so, please do not proceed with the lysis of the sample and toss the supplemented LB. Subsequently, warm up the SDC at RT and add it to a new aliquot of the not-supplemented LB. If the whiteish and cloudy solution persists, please contact our tech support (<u>techsupport@immaginabiotech.com</u>).

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB		Pag 18 of pag 20
------------	----	-------------------------	-----------------	--	------------------

App.2 Check proper beads functionalization (for Step B2 – Beads functionalization)

Comparing the difference in the absorbance measured at A 270 nm (Nanodrop ND-1000) for the unbound ligand (collected in Step B2.9) and the starting Ligand solution (collected in Step B2.3) allows an estimation of the binding efficiency. Please create a blank solution adding 2 μ L of DMSO to 50 μ L of WSS buffer and mix well by vortexing. Please utilize the blank solution just created for blank subtraction at the Nanodrop to properly quantify the Ligand from Step B2.9 and Step B2.3.

reduction in % =
$$(1 - \frac{\text{Step B2.3 A}_{270}}{\text{Step B2.9 A}_{270}}) * 100$$

Between 30% and 50% absorbance reduction in the unbound ligand compared to the starting solution is expected. If the decrease in absorbance is not observed, please incubate beads for up to 2 hours and check again the absorbance.

Sample number	Sample name	AU 290 Before Step B2.3	AU 290 After Step B2.9	Reduction %
1				
2				
3				
4				

 Table 3. Sample beads functionalization summary

		/ / /		
Mod 080262	V1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 19 of pag 20

Contacts



Info

info@immaginabiotech.com

Sale support (quoting, ordering, and order status update) orders@immaginabiotech.com

Technical service (technical inquiries and quality complaints) techsupport@immaginabiotech.com



Viale Industria, 47, 38057, Pergine Valsugana (TN), ITALY



www.immaginabiotech.com



Notes:

Mod 080262	v1	Date Exp/rev 24/01/2024	Edited by MA PB	Pag 20 of pag 20