FAQ AHARIBO

Can I use AHARIBO in animal models?

No. AHARIBO is dedicated to cell culture. The incorporation of AHA into the nascent peptides is crucial for the protocol. Despite the fact that in principle is possible to incorporate the AHA in living animals (**Sarah Calve** et al. Sci Rep. 2016; 6: 32377.) at this stage we do not recommend AHARIBO for study in animals or living organism.

I accidentally froze my beads, can I still use them?

Yes. Typically, the freezing of the beads is not recommended, but should not interfere with the beads properties.

Do I need to treat with sBlock the cell before proceeding with the lysis?
Yes, sBlock must be added to the cell to block translation and to fix the nascent peptide on the ribosome, an essential step for AHARIBO. sBlock is present also in the lysis buffer (LB) as well as in the W-buffer (WB) included in the kit. If the incubation of the cell with the sBlock is erroneously skipped, the sBlock in the lysis buffer will act stabilizing the nascent peptide on the ribosome.

How should I store the lysate for AHARIBO experiment?

We suggest for cell lysis to store them at -80°C for maximum 1-2 months.

I don't have enough time to complete all steps in a single day. Can I store the functionalized beads (after step 1) and continue later?

Yes, you can stop at step 1. We suggest keeping the beads at +4°C overnight. Do not freeze the beads.

What lysis input material can be used?

We suggest to start with a total volume of lysate corresponding to 0.2 a.u. (260 nm). On the other hands, we tested AHARIBO RNA with HeLa lysate absorbance down to 0.002 a.u. How can I calculate this absorbance?

You can simply measure the lysate at the nanodrop at 260 nm with lysis buffer as blank and use this absorbance value to calculate the volume necessary. A practical example if your lysate's absorbance (A260) corresponds to 10, this means that you have 10 a.u./mL, which then corresponds to 0.01 a.u./ µL. So, if you want to start with 0.2 a.u., 20 µL of lysate are needed.

Is there a way to improve my sample before processing in the AHARIBO kit?

Cleaning of the sample is possible to sucrose cushioning the cell lysate using standard protocols. This should remove the full length synthesized proteins, enriching the fraction of ribosomes containing the nascent chains.

For which species can I use AHARIBO?

Generally speaking, AHARIBO works well on eukaryotic ribosomes, but as of today it has been validated internally for mammalian cells and mice. sBlock being a eukaryotic specific translation inhibition should not work with prokaryotes.

How long is the kit is stable once arrived?

The kit is stable for 12 months.