FAQ RiboLace Module 1

Do I need to treat with cycloheximide the cell pellet before proceeding with RiboLace pulldown?

CHX (cyclohexamide) is present in the lysis buffer (LB) as well as in the W-buffer (WB) included in the kit.

RiboLace works well with or without cycloheximide. That being said, the choice on CHX addition to the cells depends on your experimental setup. Some users found artefacts of the P-site periodicity around the start codon due to CHX. On the contrary, CHX shift the length of the ribosome protected fragments toward a more uniform peak at 28 nucleotides. In our experience CHX at 20 ug/mL slight improves the resolution periodicity of the P-site along the coding region. But for example, good P-site periodicity was obtained from flash-freezing brain tissues, where CHX treatment was not performed.

If your sample is sensitive to ribosomes run-off we suggest to increase the CHX concentration in the W-buffer (currently 20 ug/mL, to 40 ug/mL) adding 30 ul of a CHX stock solution (10 mg/mL) to each WB bottle.

We suggest to use the protocol that suits you best, based on your experience with the biological model.

How should I freeze the sample for Ribolace experiment?

For cell lysis with CHX, we suggest for cell lysis to store them at -80°C the sample for maximum 1-2 months before performing RiboLace.

For cell lysate without CHX, we suggest to flash-freeze tissues and store at -80°C the sample for maximum 1-2 months before performing RiboLace.

For tissues, we suggest to flash-freeze tissues and store (-80°C) the sample for maximum 1-2 months before performing RiboLace.

I don’t have enough time to complete all “Day 1” steps in a single day. Can I store the functionalized beads (after step 2) and continue later?

Yes, you can stop at step 2. We suggest keeping the beads in the solution containing the RiboLace smart probe (step 2.9), i.e. before magnet separation and passivation with mPEG. After the 1h at RT - 1400 rpm you can store the beads at +4°C overnight. Do not freeze the beads.

Can I use a commercial kit for low-input small RNA-seq to prepare the library for the ribosome protected fragments?

Yes, we internally validated the use of SMARTer smRNA-Seq Kit for Illumina combined with RiboLace module 1. Some customers obtained trustable results using QIAseq miRNA

Library Kit. In general, all these protocols require a pre-step of fragments dephosphorylation that you can follow on the Protocol for RiboLace Module 2 Step 2.

Commercial small RNA-seq library prep kits generate good libraries in terms of yield and quality, but you will lose a little bit of periodicity along the CDS.

What cell/RNA input material can be used?

RiboLace works well with 100,000-3M cells (70-80% confluence). Some customers obtained good results also with 10,000 cells, but the number of cells to use depends strongly on the type of cells and studied diseases/conditions. That's because RiboLace isolates only ribosomes that are actively translating (avoiding noise from stalled ribosomes). If your cells are translationally depressed (e.g. due to drugs, heat shock treatment, or some particular disease) you probably will need more cells to get a good result at the end. Speaking about total RNA amount: we suggest between 0.1 and 2 ug of total RNA.

For Protocol Module 1, you suggest to start with a total volume of lysate corresponding to 0.03 a.u. (260 nm). 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 0.3 a.u., 30 µL are needed.

Is there a way to concentrate my sample before processing in the RiboLace kit?

You can try to lysate the cell with less lysis buffer (LB) (0.05 ml instead of 0.3 ml). The only concern is that such a low amount of LB in a 100mm dish could make difficult to obtain an efficient cell lysis. In this case you can try to seed your cells in dishes with lower surface-area i.e. 6-well plate.

For which species can I use RiboLace module 1?

Generally speaking, RiboLace works well on eukaryotic ribosomes, but as of today it has been validated internally for mammalian cells, mice and insect tissues. At the moment we are working on validation experiments on plants and prokaryotes.

In Step 6, should I add acid phenol:chloroform:isoamyl alcohol to the bead suspension?

Yes, you should. In fact, your ribosomes are still attached to the beads after Step 5 (washing).

How long is the kit stable for once arrived?

The kit is stable for 12 months.