Sample Submission for RNA Research (Cells, Tissues and FFPE Tissues)

We recommend our customers to process the samples according to the protocols listed below. If you use other protocols, please provide us as much detail as possible on your method prior sample submission.

Note: RNA samples are very sensitive to RNase digestion; therefore, wear gloves, use RNase-free disposables and maintain an RNase-free work area.

Cells

Recommended sample amount: 2 x 10^6 cells each sample.*
**For MeRIP-sequencing, the recommended amount is 1-2 x 10^7cells each sample.*

Suspension cells

1. Harvest cells and pellet cells by centrifugation. Use 1 ml of the TRIzol Reagent per 5-10 x 10^6 cells.
2. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of TRIzol Reagent to avoid any RNA degradation.
3. Store at -80°C.
4. Ship on dry ice.

Adherent Cells

1. Lyse cells directly in a culture dish by adding 1 ml of TRIzol Reagent to the dish and passing the cell lysate several times through a pipet tip. The amount of TRIzol Reagent required is based on the culture dish area (1 ml per 10 cm^2) and not on the number of cells present.
2. Store at -80°C.
3. Ship on dry ice.

Tissues

Recommended sample amount: 10-25mg tissue each sample.*
**For MeRIP-sequencing, the recommended amount is over 50mg tissue each sample.*

A. Prepare using RNAlater Reagent

1. Excise the tissue sample from the animal/patient and, if necessary, cut it into slices less than 0.5 cm thick. Perform this step as quickly as possible and proceed immediately to step 2.
2. Completely submerge the tissue piece(s) in the collection vessel containing RNAlater RNA Stabilization Reagent. At least 10 volumes of the reagent (or approximately 10 µl reagent per 1 mg of tissue) is required.
3. Incubate the tissue overnight in the reagent at 2-8°C. Then store at -20°C.
4. Ship at 4°C

B. Prepare using TRIzol Reagent

1. Homogenize tissue samples in 1 ml TRIzol Reagent per 10-25 mg tissue using a tissue homogenizer. The sample volume should not exceed 10% of the volume of TRIzol Reagent used for homogenization.

2. Store at -80°C.
3. Ship on dry ice.

C. Freeze in liquid nitrogen

1. Wash the fresh tissue with 1x PBS and, if necessary, cut the tissue into small pieces. Perform this step as quickly as possible and proceed immediately to step 2.
2. Freeze the tissue in liquid nitrogen for 2-3 hours.
3. Store at -80°C or in liquid nitrogen.
4. Ship on dry ice. Alternatively, grind the tissue samples to powder in liquid nitrogen, followed by homogenization in Trizol, then ship at 4°C or on dry ice.

FFPE sample

1. The quality of RNA isolated from FFPE samples will depend on the technique used to preserve the sample and on other factors such as the age of the block.
2. List below is the standard fixation protocol that is best suit for gene expression analysis:
   (1) Fixate tissue sample in 4-10% formalin immediately after surgical removal
   (2) Use a fixation time of 14-24 hours
   (3) Completely dehydrate the samples prior to embedding in paraffin
3. Ship at room temperature.