

## TU-RNA BIOTINYLATION

- REAGENTS:  
**RNase free 10x Biotinylation Buffer (BB)**  
100 mM Tris pH 7.4  
10 mM EDTA  
=> store in aliquots of 1 – 1.5 ml at 4°C
- **Biotin-HPDP** (Pierce, 50mg EZ-Link Biotin-HPDP, Cat. Nr. 21341)
- Stock concentration: 1 mg/ml dissolved in Dimethylformamide (**DMF**) store aliquots of 50 µl, - 20 °C

### PROTOCOL:

#### **Labeling Reaction (use 30 – 100 µg total RNA):**

- 2 µl Biotin-HPDP (1mg / ml DMF) per 1 µg RNA
- 1 µl 10x Biotinylation Buffer per 1 µg RNA
- 7 µl RNase free H<sub>2</sub>O per 1 µg RNA
- Incubate at room temperature for 1.5 h with rotation.
- Add an equal volume of Chloroform/Isoamylalcohol (24:1).
- Mix vigorously. Incubate for 2 – 3 minutes until phases begin to separate and bubbles start to disappear.
- Centrifuge at full speed (20,000 g) for 5 min, room temp.
- Carefully transfer upper phase into new tubes.
- Repeat step using Phase-Lock gel tubes. To further reduce RNA loss we perform the second chloroform extraction using Phase Lock Gel Heavy tubes (2.0 ml, Eppendorf) following the manufacturer's instructions.
- In principle, a single chloroform extraction step is enough to remove virtually all unincorporated Biotin-HPDP. Still, we perform two rounds to ensure complete removal. Usually we only use the phase-lock tubes for the second round as 1 ml biotinylation volume is too much for these tubes. After the initial chloroform extraction only about 80% of the volume remains as the DMF is also removed.
- **RNA precipitation:**  
Add 1 /10 the reaction volume of 5 M NaCl
- Add an equal volume of isopropanol and centrifuge at 20,000 g for 20 min 4°C
- Remove supernatant.
- Add an equal volume of 75% ethanol
- Centrifuge at 20,000 g for 10 min.
- Resuspend RNA in 1.0 µl RNase-free H<sub>2</sub>O per mg starting RNA

#### **Comments:**

The chloroform extraction is required to remove unincorporated biotin-HPDP. To reduce the loss due to the extraction procedure the initial volume should be at least 500 µl. Smaller volumes should be increased by the addition of 1x TE.