RNA ISOLATION

Main mix: (cover with aluminium foil):

4M guanidinisothiocyanat	**20 ml**
25 mM sodium citrate pH 7	**500 µl**
0,5% Sarcosyl	**100 µl** (300 µl of 30% Lauroylsacosine Sodium salt solution)
0,1 M 2-mercaptoethanol	**140 µl**

Final Mix: 1ml main mix + 0,1 ml 2M sodium acetate + 1 ml Phenol (water saturated)

For 40 ml (prepare under the fume hood)
20 ml mix
2 ml 2M Sodium acetate
20 ml Phenol

Use 100 ml PET bottle (RNase, DNase free)

4M guanidinisothiocyanat:	1M sodium citrate pH 7	2M sodium acetate pH 4
4.72g/100 ml DEPC-H2O	2.941g/100ml DEPC-H2O pH 7 (tri-Natriumcitrat-Dihydrat OK)	2.722g/100ml DEPC-H2O

Use RNase free eppis!

- For cells growing in a dish, take away the medium and wash with 1x PBS
- Add 1 ml Mix per 10 cm^2
- Incubate 5 min at RT. Distribute in 1ml fractions in eppis.
- Add 0.2 ml Chloroform.
- Shake per hand for 15 sec. and incubate 3-10min at RT.
- Centrifuge 5 min, 13 000 rpm, RT.
- Upper liquid phase > RNA, Interphase > DNA, Phenol/Chloroform Phase > Proteine
- Transfer the upper liquid phase to a new tube
- Add 0.5 ml Isopropanol
- Mix well
- Incubate 5-15 min on ice (4°C) or at -20°C
- Centrifuge 10 min, 13000 rpm, 4°C
- Remove supernatant
- Wash 2x with 75% ethanol (4°C)
- Centrifuge 5-10 min, 13000 rpm, 4°C
- Air dry the pellet
- Resuspend the pellet in RNase free water

Denaturing Agarose Gel Electrophoresis of RNA

- Add 0.6g of Agarose in 33,5 ml of ddH2O. Boil until agarose is dissolved (use LE GP Agarose of Biozym, for 300 – 7000 bp)
- Let it cool down a bit, place it into a fume hood
- Add 5 ml 10X MOPS buffer and 9 ml of Formaldehyde 37% and 7 µl of 10000x SyBr Safe.
- Let cool down until it can be comfortably held.
- Set up the Agarose gel casting tray in the fume hood.
    - Wash with 1% SDS, DEPC water, RNase Away
- Prepare RNA sample (1 µg per lane is enough) using RNA sample buffer (2X Fermentas) + SyBr safe (for 5 mm lane, use at least 3 µl RNA ladder).
- Heat 10 min to 70°C (Fermentas protocol) and place on ice.
- Remove the dams from the gel casting tray and cover the gel with 1X MOPS buffer.
- Load the sample (use at least, 3 ul RNA ladder for 5 mm lane + load at least 500 ng sample).
- Run the gel at 100V for 1.5hr or until 80% of the gel length (RNA is negatively charged and migrate towards the positive electrode!)
- Let the gel casting tray + buffer etc … under the fume hood for a couple of days before washing.

10X MOPS buffer (1L):		**(500 ml)**
MOPS	41.86 g	100 ml (1M)
Sodium Acetate	6.80 g	12.5 ml (2M)
0.5 M EDTA (EDTA = Titriplex)	20 mL	10 ml (0.5M)
DEPC-H2O to	1000 ml	500 ml
pH 7 with NaOH		

protect from light at 4°C

1M MOPS:
209.3g / 1L DEPC-H2O

0.5M EDTA:
186.12 g / 1L DEPC-H2O
Adjust pH to 8 with NaOH

Use a clean 100 ml/500 ml PET bottle.