

## SOP 3.8.2 RNA Extraction from Cells in Urine

SOP Number: 3.8.2  
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

### Purpose

This SOP describes the procedure for RNA extraction from cells in urine.

### Responsibility

It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

### Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient solution
- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- RNase free water
- An incubator/water-bath capable of generating a temperature of 65°C
- A refrigerated centrifuge capable of 12,000g
- A vortex mixer
- -80°C Freezer
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop
- BioRad Experion/ Agilent Bioanalyser 2100

### Procedure

1. Resuspend the cell pellet from step 4 in SOP 3.8 in 20mL 1X TRI reagent. Store the homogenate for 5min at RT (18-22°C).
2. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15min at 4°C.

3. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8min at 4-25°C.
4. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5min at 4-25°C.
5. Solubilisation: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µl of FORMAZol, 0.5%SDS, or DEPC treated RNase free water and incubate at 55-60°C for 10min.
6. The absorbance of the RNA at 260nm and 280nm should be measured using quartz cuvetts or the nanodrop method to assess purity. The 260/280 ratio should be >1.8. An  $A_{260}$  of 1.0 in a 1-cm light path is equivalent to a RNA concentration of 40µg/mL. The RNA sample is aliquoted and stored at -80°C.
7. An aliquot of the RNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel in denaturing conditions. Briefly the RNA solution should be diluted with RNA loading buffer at a ratio of 1:2 and heated at 65°C for 10min and loaded into the wells in the gel and electrophoresed for 40min at 60V. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.
8. Total and/or mRNA quality and quantity can also be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality.

**Note:** There are a number of commercially available kits for RNA extraction from different manufacturers. When using these methods follow the manufacturer's instructions as outlined in the information for use/ package insert included with the kit. The method used for RNA extraction should be recorded in the study specific documentation or data management system.

**Change History**

<b>SOP Number</b>	<b>Effective Date</b>	<b>Significant Change</b>	<b>Previous SOP No.</b>