



RNA EXTRACTION FROM NASAL WASHES

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BACKGROUND

- For isolation of RNA from a small number of cells like in Nasal Wash Samples

NOTES

- Work on RNA bench and use clean pipettes, tips, gloves, use RNase ZAP spray

EQUIPMENT

- Equipment:
 - o Vortex
 - o Centrifuge
 - o Heat-block
 - o Pipettes and Tips, Tubes
- Reagents:
 - o Lysis Buffer with mercapto-ethanol
 - o Dry ice
 - o RNAqueous-Micro Kit from Ambion, Cat# 1931, 50 preps

PROTOCOL

- o Harvest nasal washes in lysis buffer and mercapto-ethanol (350 ul) and freeze on dry ice, store in -80°C
- o Thaw on ice and follow the instructions from booklet http://www.ambion.com/techlib/prot/fm_1931.pdf
- o Add **0.5 volumes of 100% ethanol, vortex** briefly and **spin** to collect all the sample at the bottom of tube
- o **Load** the lysate/ethanol mix (up to 150 ul) **onto Micro Filter Cartridge Assembly**
- o **Centrifuge for 10 sec.** at 13.400-15.500xg or 12.000-13.200 rpm; repeat until all sample has passed through the filter
- o **RNA is now bound to the filter**
- o Add **180 ul** of reconstituted **Wash solution 1**, centrifuge for 10 sec.
- o Add **180 ul** of rec. **Wash solution 2/3**, centrifuge, repeat a second time with same amount
- o **Empty collection tube** and **centrifuge for 1 min** to dry the filter
- o Label Micro Elution Tube and apply **7 ul** preheated **elution solution** to center of filter, keep at **room temp. for 1 min. Centrifuge for 30 sec.** to elute RNA
- o Repeat again with **6 ul elution solution** in the same tube (dead volume of around 2 ul stays in the filter)

DNase treatment and DNase inactivation

- Add **1/10 volume of 10x DNase I buffer** (eg. eluted in 13 ul, add 1.3 ul of buffer) and **1 ul of DNase I**, mix gently
- Incubate for **20 min at 37°C** (in meantime thaw DNase Inactivation Reagent at room temp)
- Vortex DNase Inactivation Reagent, **add 2 ul**
- Transfer to a small tube, in big tube the pellet of DNase Inactivation Reagent can't form properly
- **Centrifuge for 1.5 min to pellet the DNase Inactivation Reagent**
- **Transfer to fresh RNase-free tube**
- **Spec RNA**, use elution buffer from the kit for blank on the spec
- **Freeze at -80°C**

LINKS AND REFERENCES

http://www.ambion.com/techlib/prot/fm_1931.pdf