Standard Operation Procedure (S.O.P.) for cRNA Purification using the MEGAclear Kit

S.O.P. prepared by Andrea Rodríguez, 2021
Modified by José E Lizardi, Ph.D., 2022

This S.O.P. will direct you in the procedure of cRNA purification from enzymatic reaction, such as *in vitro* transcription, through the MEGAclear Kit (cat. no. AM1908). This kit can also be employed to purify antisense RNA amplifications (aRNA). The afore mentioned method provides a simple and fast way of efficiently removing nucleotides, short oligonucleotides, proteins, and salts from RNA. The kit is appropriate for single-stranded RNA larger than 100 nt and double-stranded RNA larger than 200 bp, and it recovers from 1 ng to 500 μg of RNA with a 70% or higher efficiency. The purified cRNA could be employed in applications that requires high purity cRNA. In our case, the cRNA will be microinjected in *Xenopus laevis* oocytes.

The Elution Solution's composition is 0.1 mM EDTA (pH 8) in nuclease-free water. However, the eluted cRNA can be directly microinjected in X. laevis oocytes without any other manipulation.

***Important*:**

- ***Check if ethanol (100%) was added to the Wash Solution (a box in the side label should be checked). If not, add 20 mL of ethanol before use.***
- ***It is recommended to clean the bench and pipettes with an RNase decontamination solution. Use 70% alcohol if decontamination solution is not available.***
- ***The Spin Column is made up of two parts: the Filter Cartridge (clear tube with a white silica membrane band) and the Collection Tube (clear).***
- ***The Elution Tube is the same than the Collection Tube.***
- ***Microcentrifuge must be capable of attaining 10,000–15,000 x g.***
- ***Filter Cartridges should not be subjected to over 16,000 × g for risk of mechanical damage and/or glass contamination in the sample.***
- ***All tips and microtubes must be autoclaved (RNase free).***
- ***All volumes are given on a per culture tube basis.***

Materials:

- 70% Alcohol or RNase Decontaminant (RNaseZap)
- Waste Container with Detergent (1% Alconox, stored at room temperature)
- Micropipettes and Tips for 100, 200, and 1000 μL
- 100% Ethanol
- 70% Ethanol
- 5 M Ammonium Acetate
- Binding Solution (stored at 4 °C)
- Wash Solution (stored at 4 °C)
- Elution Solution (stored at 4 °C)
- Spin Column (Filter Cartridges + Collection Tubes)
- Elution Tube
- Dry Bath at 95 °C
- Microcentrifuge for 1.5 mL
- Vortex

Procedure:

a) The working area should be sanitized using RNase decontamination solution or 70% ethanol prior to placing any materials on the bench. Prepare the waste container with 1% alconox.

b) Pre-heat 55 µL of Elution Solution in the dry bath at 95 °C.

c) Add Elution Solution to the cRNA sample to bring the cRNA mixture to 100 µL. Vortex gently.

d) Add 350 µL of Binding Solution to the cRNA mixture. ***Mix gently by pipetting***.

e) Add 250 µL of 100% Ethanol to the cRNA mixture. ***Mix gently by pipetting***.

f) Transfer the cRNA mixture to the Spin Column and centrifuge at 15,000 x *g* for 1 minute.

g) Remove the Filter Cartridge from the Spin Column and discard the liquid in the Collection Tube into the waste container. Return the Filter Cartridge to the Collection Tube.

h) Wash the Filter Cartridge by adding 500 µL of Wash Solution and centrifuge the Spin Column at 15,000 x *g* for 1 minute.

i) Discard the Wash Solution as explained above (***step f***) and add 500 µL of Wash Solution to the Filter Cartridge for a second wash.

j) Centrifuge the Spin Column at 15,000 x *g* for 1 minute, discard the Wash Solution as explained above (***step f***), and centrifuge the Spin Column again for 30 seconds at 15,000 x *g*.

k) Elute cRNA from the Filter Cartridge as follow,
   1) Transfer the Filter Cartridge into a new Elution Tube.
   2) Apply 50 µL of **Elution Solution** to the center of the Filter Cartridge. Close the cap of the tube and centrifuge for 1 minute at 15,000 x *g* at room temperature to elute the cRNA.
   3) *Optional*: Apply another aliquot of 50 µL of **Elution Solution** and repeat the centrifugation to maximize RNA recovery. Collect the eluate into the same Elution Tube.
   If glass fibers are observed in your sample, they can be removed by centrifuging the sample briefly and then transferring the RNA to a new tube.

l) ***Optional*****:** Precipitate with 5 M Ammonium Acetate to concentrate cRNA.
   1) Add 1:10 volume of 5 M Ammonium Acetate to the purified cRNA (*i.e. If sample was eluted with 50 µL of Elution Solution, 5 µL of 5 M Ammonium Acetate will be added*).
   2) Add 100% ethanol at a volume that is 2.5 times that of the elution (i.e. If sample was eluted with 50 µL of Elution Solution, 137.5 µL of ethanol will be added), vortex gently, and incubate at –20 °C for 30 minutes.
   3) Microcentrifuge at 30,000 x *g* for 15 min at 4 °C or room temperature, preferably at 4 °C.
   4) Carefully remove and discard the supernatant.
   5) Wash the pellet with 500 µL of cold 70% ethanol, centrifuge again at 30,000 x *g*, and carefully remove the ethanol.
   6) To remove the last traces of ethanol, quickly recentrifuged at 30,000 x *g*, and aspirate any residual fluid with a very fine tipped pipette or with a syringe needle.
   7) Air dry the pellet.
   8) Resuspend the pellet using the desired solution and volume

m) Decontaminate all the equipment that was used with 70% ethanol. Carefully discard the alconox solution in the waste container into the proper waste container and discard the materials in a biological waste (trash can with red bag).

n) Be sure all equipment are shut down. Return any material and equipment to its proper place. The pipettes must be returned to their maximal volume.