Purifiy HDV RNA by FPLC

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Adapted from Andrew Knappenberger

# Materials

H2O (2L)

20% EtOH (2L)

* Molecular use EtOH 200ml
* D.D. water 800ml
* Without filter

0.1M NaOH (1L)

* 4g NaOH
* Dissolve 4g NaOH in 1L H2O
* Filter

5X Gel filtration buffer stock (2L)

5X concentration

|  |  |
| --- | --- |
| Item | Concentration |
| MES pH. 6.2-6.3 | 250mM |
| KCl | 500mM |
| MgCl2 | 50mM |

1X working concentration

|  |  |
| --- | --- |
| Item | Concentration |
| MES pH. 6.2-6.3 | 50mM |
| KCl | 100mM |
| MgCl2 | 10mM |

Recipe for 5X stock

|  |  |  |
| --- | --- | --- |
| Item | MW (g) | Add (g) |
| MES | 195.2 | 97.6 |
| KCl | 74.55 | 74.6 |
| MgCl2 | 203.3 | 20.3 |
| Dissolve in 1.8L D.D. H2O | | |
| Adjust pH to 6.2-6.3 | | |
| Add water to 2L | | |
| Filter the solution and store at 4oC no longer than 3 months. | | |

Amicon Ultra-15 Centrifugal Filter Units

Use 10KDa one.

<https://www.emdmillipore.com/US/en/product/Amicon-Ultra-15-Centrifugal-Filter-Units,MM_NF-C7715?ReferrerURL=https%3A%2F%2Fwww.google.com%2F>

# Procedure

## Concentrate and buffer exchange the transcribed RNA

1. Use an Amicon filter with 15 mL sample size and 10 kDa cutoff to concentrate the RNA and exchange its buffer to gel filtration buffer. All spins should be 10 mins at 4,000 xg. See the manual for details.
2. Spin the transcribed RNA through once to reduce the volume. Add 10 mL of gel filtration buffer and spin again to buffer exchange. Use Beckman rotor to centrifuge at 4000 rpm for 15min.

## Purify the RNA

### On the day before you plan to do FPLC

DO NOT link the column until further notice

1. Run 0.1 M NaOH through the FPLC (not the columnn) until the conductivity reaches a new and constant level.

Any speed that does not exceed pressure limits is fine. (~5 mL/min)

Reason: The NaOH is primarily to remove residual RNAse contamination from any previous protein purifications.

1. Let the system sit with NaOH for another 30-45 mins.
2. Run 2 column volume (CV) dH2O

Now you can link the column

1. Then 2 CV gel filtration buffer **over through the column**. This step can be done overnight.

\*CV: Column volume

### On the day for FPLC

DO NOT link the column until further notice.

!!Whenever you use the syringe, take care that you do not introduce any air into the FPLC.!!

1. Take a fresh syringe and the "RNA Only" needle.
2. Load the syringe by pouring liquid into the top rather than drawing things up the needle.
3. Since the needle is likely to be dirty, flush it 3X with 0.1 M NaOH.
4. Load ~2.5 mL 0.1 M NaOH into the sample loop and run it through the system, **bypassing the column.**
5. Flush the syringe and loop with dH2O the same way.
6. Finally, do the same with gel filtration buffer.
7. Then run the FPLC, **bypassing the column**, for about 30 mL.
8. Stop the FPLC.

Now you can link the column

1. Load the sample.

The sample is loaded in much the same way as if flushing the sample loop. Once the sample is in the sample loop, you can leave the room and start the method file remotely.

1. Fill the fraction collector with an appropriate number (28) of 13 x 100 mm glass tubes.
2. Run the method:

Settings: from Joseph D. Puglisi’s paper

Flow rate is 3mL/min At 4oC by collecting 8 ml volumn

1. After 1.2 CV, the system is ready for purifying another RNA.

Therefore, it is highly advantageous to do purification for all constructs sequentially in one session.

1. To flush the system in between samples:
   1. Flush the sample loop with buffer using the system until the UV absorbance flatlines.
   2. Then clean the syringe and needle twice with buffer.
   3. Fill the syringe with buffer and inject the contents into the sample loop.
   4. Flush the sample loop with buffer again. If there is a peak, repeat the cleaning procedure until the peak disappears.

## Pool and concentrate the purified RNA

1. Inspect the results of running the method file. Pool all fractions that are likely to contain pure RNA monomers.
2. Concentrate the RNA as above. Keep it in gel filtration buffer for storage; or exchange into an appropriate buffer (e.g., equilibrium dialysis buffer) when conducting an experiment.

If you know your RNA will not aggregate, you can jump step 20.

1. Take a sample of the purified RNA (how much?) and run it on the FPLC as before. Use "HiLoad Superdex 75 pg RNA gel filtration no frac".

Here, we won't be collecting fractions. Instead, we'll be looking to see whether the purified RNA monomer again forms aggregates or does anything else that would make it more heterogeneous in crystallization conditions.

## Post Run and Wash column

1. After completing all runs, wash the FPLC and column with 2 CV dH2O and 2 CV 20% EtOH. This step can be done overnight.