Histone separation on FPLC

Revised by C Pham
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Procedure

Sample loop: 500ul or less
Column: C-18 column
Make sure all connections are tight.

1. When your column is not mounted on the FPLC, transfer tubings A1 and B1 in H2O.

2. Purge the four valves
   2.1. Use about 10-20ml/valve using the provided syringe.
   2.2. Go to Manual, pump, pumpwash purifier.
   2.3. Click on A1 and B1.
   2.4. Click execute.
   The wash will stop automatically.

3. Wash fraction collector.
   3.1. Go to Manual, flow rate: 5ml/min, fraction: Fraction900, 50ml,
   3.2. Click execute.
   Run 10min.
   3.3. Click end to stop it.

4. Wash the waste tubing as above.

5. Transfer tubing A1 to aqueous buffer, B1 to organic buffer.

6. Purge valves as above.

7. Connect the head of the column to the orange tubing.

8. Wash fraction collector with 70%B buffer.
   8.1. Go to Manual, flow rate: 1ml/min, gradient: 70%B, 1 min, fraction: Fraction900, 50ml, pressure limit: 15Mpascals
   8.2. Click execute.
   8.3. Put the collector tubing to the wash bottle.
   If you see liquid come out from the other end of the column, connect that end to the flow cell.
   Wash the column for 30 minutes to 1 hour until the baseline is stable.

9. Wash column with 0%B buffer
    9.1. Go to Manual, flow rate: 1ml/min, gradient: 0%B, 1 min, fraction: Fraction900, 50ml, pressure limit: 15Mpascals.
    9.2. Click execute.
    9.3. Put the collector tubing to the wash bottle.
    Wash around 30min to 1 hour until the baseline is stable.

10. Label tubes and put them in the fraction collector.

11. Prepare sample
    11.1. Put on the sample loop, and wash it with 5-10 volumes of water.
    11.2. Spin histone sample at maximum speed for 2 minutes at 4C.
    11.3. Transfer sample to the sample loop using a 1ml disposable syringe, transferring the same volume as the sample loop.

12. Pull out your run method on histones.
    Before you click run, put the fraction collector arm to tube 1. Click run.

13. If you need shoot more samples, just repeat steps 5 to 10. Just leave the column on.

14. When you're done using the column, transfer tubings A1 and B1 into a solution of 70% acetonitrile/30% water.
15. Wash the column
    15.1. Go to Manual, flow rate: 1ml/min, fraction: Fraction900, 50ml, pressure limit:15Mpascals
    15.2. Click execute
    15.3. Put the collector tubing to the wash bottle.
    Wash for 1 hour until the baseline is stable.

16. Disconnect the column from the FPLC and cap the column. Put it away.

17. Wash the sample loop 5-10 volumes of the sample loop with water.

18. Speed vac your fraction tubes and resuspend them in water. Run on a gel.

19. Wash the FPLC. See "Washing the FPLC" instructions.

Solutions

Buffers need to be filtered and degassed.
Use a filter for organic solutions. Wet in 100% MeOH first and then quickly rinse in water. Filter the organic buffer B first and then aqueous buffer A. You can use the same filter membrane.

Aqueous buffer(A)

50ml(5%) Acetonitrile
1ml(0.1%) Trifluoroacetic acid
H2O to one liter

Organic buffer(B)

900ml(90%) Acetonitrile
0.92ml(0.92%) Trifluoroacetic acid
H2O to one liter