AB108.1
Modified 5.4.23 by KCB

Purification of Monoclonal Antibody by Melon Gel

Materials and Reagents:

1. Melon™ Gel IgG Purification Kit (ThermoFisher Catalog number: 45212)
    2. Melon Gel IgG Purification Slurry
    3. Melon Gel Purification buffer (makes 1L of 100X buffer)
    4. Melon Gel Regenerant (makes 1L of 5X buffer)
5. Hybridoma culture supernatant (from AB100.6)
6. Vacuum filter bottle set, top and bottom
7. Vacuum tubing
8. Vacuum source
9. Spatula
10. 100kDa Amicon spin column, 70 mL
11. Glass screw-top bottle (wash bottle)
12. Dialysis Tubing 12-14kDa (optional)

Protocol:

1._____ Equilibrate the Melon gel slurry, purification buffer, and regenerant to room temperature, about 30 minutes.

2._____ Concentrate hybridoma cell culture supernatant to a minimum of 100 mL using a 100kDa Amicon spin column, spinning at 3500g until the desired volume is reached. (Note 1)

3._____ Perform a buffer exchange with the same Amicon by washing the sample 3 times with 1X Melon gel purification buffer and spinning after each wash or dialyze the supernatant with two 1-hour exchanges of 1X Melon Gel Purification Buffer at room temperature. (Note 2)

4._____ Set up the vacuum filter bottle by hooking up the vacuum hose to the bottle and any vacuum source.

5._____ Wet the membrane of the vacuum bottle with either MilliQ water or 1X Melon gel purification buffer.

6._____ Gently swirl the Melon gel slurry until you obtain an even suspension, and the mixture is uniform (do not vortex).

7._____ Add a volume of slurry equal to the volume of the sample to the top half of the vacuum filter bottle, on top of the membrane. (Note 3)

8._____ Using the wash bottle below, apply a vacuum to drain the storage buffer from the Melon gel. Avoid over-drying the gel, as it could result in a sub-optimal yield.

9._____ Add the hybridoma culture supernatant in 1X purification buffer and gently mix with the spatula.

10._____ Incubate the mixture for 5 minutes at room temperature.

11._____ Transfer the filter top containing the slurry to a new bottle.

12._____ Apply vacuum to collect the purified antibody, avoiding over-drying the Melon gel.

13._____ Wash the Melon gel by applying another volume of 1X antibody purification buffer equal to the volume of the gel-bed and mix with the spatula. Incubate at room temperature for 5 minutes and pull the wash through the filter into the same bottle.

14._____ Repeat the wash (step 11) two additional times for a total of 3 washes.

15._____ Re-concentrate the purified antibody using a 70 mL amicon spin column down to its original volume by spinning at 3500g until the desired volume is reached.

16._____ Regenerate the Melon gel by 1.5 times the gel-bed volume of 1X Melon gel regenerant buffer and stir for 30 seconds with the spatula. Incubate for 5 minutes at room temperature.

17._____ Switch the screw-cap bottle to the wash bottle, and apply vacuum to drain the Melon gel, avoiding over drying the gel.

18._____ Wash the gel with 10 times the gel-bed volume of MilliQ water

19._____ Re-equilibrate the Melon gel by washing with 10 times the gel-bed volume of 1X Melon gel purification buffer.

20._____ Repeat steps 8-18 2 more times, for a total of 3 passes over the melon gel, or until pure antibody is achieved.

21._____ Assess purity by SDS-page and concentration of the purified antibody by BCA. (Note 6)

22._____ Buffer exchange into phosphate buffered saline, pH 7.2 or tris-buffered saline pH 7.2 for long term storage. (optional)

Notes:

1. The maximum volume recommended for purifying in a single batch is 100mL. Samples lower in volume than 100mLs can be processed as-is. Additionally, the supernatant can be concentrated using an ammonium sulfate cut of 50%, however, it could affect the integrity of the antibody.
2. If exchanging buffers by dialysis, the volume of 1X Melon gel purification buffer used should be at least 50 times the volume of the culture supernatant. Equalize the 12-14kDa dialysis membrane in MilliQ water for 30 minutes before adding the hybridoma culture supernatant.
3. If the sample was concentrated in any way with a protein cutoff of less than 75kDa, then up to 5 times more slurry may be needed to purify the antibody. Melon gels work to purify the antibody by binding everything else in the media, so if other contaminants were concentrated as well, more slurry will be needed.
4. For storage, wash column with 10 times the gel-bed volume of 1X Melon Gel Purification Buffer. For storage longer than 1 week, add a final concentration of 0.02% sodium azide to the buffer used to wash the column.
5. For the batch format, 200mL of settled gel has the capacity to purify up to 1L of cell culture supernatant containing up to 10% FBS. Typically, the gel can be regenerated five times without significant loss of selectivity.