

PROTOCOL FOR GLYCEROL REMOVAL

Protocol for removal of glycerol from the storage buffer of Triple A Polyclonals™ and PrecisA Monoclonals™ from Atlas Antibodies.

Glycerol can be removed using Amicon Centrifugal Filter Units, 10 kDa cutoff (Merck Millipore UFC501096). The filter unit concentrates the antibody sample during centrifugation by allowing the buffer to flow through the membrane but stops any proteins above 10 kDa from passing.

The antibody is then resuspended in a buffer of choice, thus allowing for buffer exchange. Consider testing the antibody +/- the glycerol cleanup, to see whether there is an improvement. Some antibodies perform better even if there is glycerol in the buffer

PROCEDURE

1. Dilute the antibody to 400 µl with sterile PBS (or other buffer of choice).
2. Place the Amicon filter unit into the supplied collection tube.
3. Add 400 µl of diluted antibody to the filter unit. Close the lid.
4. Spin for 5-10 minutes at 14k*g or until 100 µl of the antibody sample remains in the spin column. If this has not been achieved during the first 5 min, then centrifuge for a few more minutes until only 100 µl remains. Please note that the suggested time it takes to concentrate the sample down to 100 µl should be seen only as a guideline and will ultimately have to be determined by the user. For all spins, place the filter part of the filter unit and the elbow of the collection tube towards the centre of the rotor. All spins are performed at room temperature.
5. Discard the flow through.
6. Add 300 µl of sterile PBS (or other buffer of choice) to the filter unit and centrifuge again as outlined in step 4. Then discard the flow through.
7. Repeat step 6 four more times until the glycerol content is 0,02 %. 5-minute centrifugations are usually enough to reach 100 µl left in the filter unit at these steps. In the last round, centrifuge for 4 minutes until 100 µl is reached.
8. In new collection tube, place spin column upside down.
9. Spin for 2 min at 1k*g to collect buffer-exchanged antibody. Store antibody at 4 °C until needed.

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