

PRODUCTION OF ANTIBODY AGAINST GLOMALIN FROM THE 32B11 CELL LINE

(Dazzo and Wright, 1996; Goding, 1986; Hurrell, 1982; Wright, 1994; Wright *et al.*, 1996; Zola, 1987)

The B cell line for production of the antibody against glomalin (32B11) can be purchased from ATCC (American Type Culture Collection). The catalog number is CRL-2559. The cell line should be stored in liquid N₂. After culturing, the serum containing the 32B11 antibodies may be used in immunoreactive assays, such as ELISA, immunodot blot, and immunofluorescence, to quantify and identify the location of glomalin.

Culture Media Production

RPMI media supplemented with 10% Fetal Calf Serum

Makes 500 ml. **Note: All reagents including the water must be tissue culture grade.**

Materials

1. 8.2 g RPMI medium (HEPES)*
2. 0.03 g penicillin**
3. 0.066 g streptomycin**
4. 0.055 g sodium pyruvate
5. 1.0 g sodium bicarbonate
6. 400 ml DD H₂O (T 15-20 °C)
7. 50 ml FCS (Fetal Calf Serum) for 10% FCS in final solution***
8. Sterivex (GV 0.22um filter unit)
9. 500 ml tissue culture flasks (preferably with 0.22 um filters in top)
10. 70% EtOH

*Glutamine in RPMI medium has a short half-life. If you are storing media longer than 15 days, add 0.30 g/L of L-glutamine by suspending L-glutamine in as small amount of DD H₂O as possible then filter into flask before use. Note: It is best to use media within a few days.

**Antibiotics prevent bacterial growth in the media, but sterile techniques must be adhered to or the sample will become contaminated by fungi. This is a very nutrient rich media and is easily contaminated.

***FCS must be kept in the dark (light can result in the formation of toxins). FCS will have some insoluble material in it, but if it looks cloudy, you will have to filter before using (changing filters frequently).

Method

1. Rinse graduated cylinder or beaker and stir bars thoroughly with Milli-Q water before making media.
2. Combine #1-6 and add ice cold DD H₂O* until volume reaches 450 ml. Mix completely (Fig. 1).

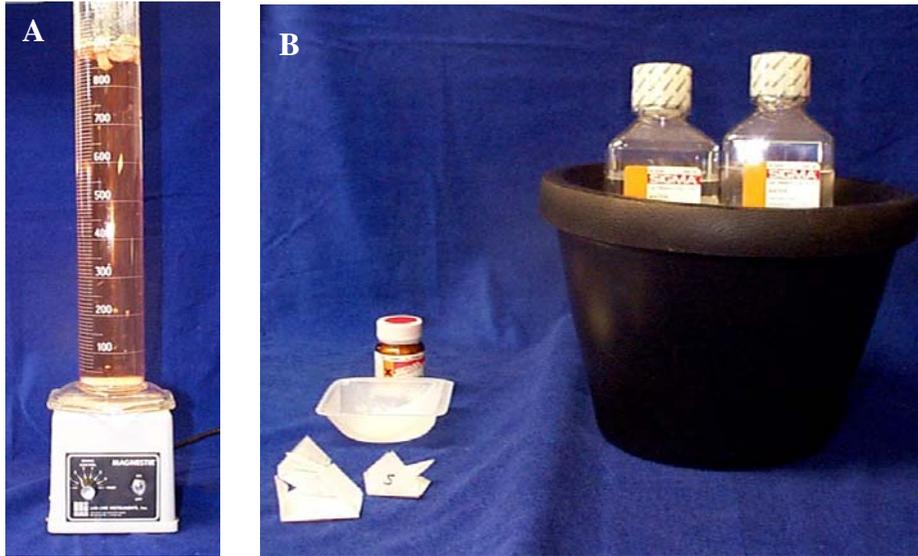


Figure 1. All ingredients in the RPMI media are mixed together, except for the Fetal Calf Serum (A) and brought to volume with ice-cold tissue grade water (B), which also helps to dissolve some of the amino acids.

3. Filter sterilize through a 0.22 μ m filter unit using a peristaltic pump into sterile tissue culture flasks (Fig. 2). Sterile techniques must be adhered to including working under a laminar flow hood or in a biosafety cabinet with gloves and sterile sleeves for your arms.



Figure 2. The RPMI media is filtered through a 0.22 μ m filter into a tissue culture flask before adding the Fetal Calf Serum.

4. Sterilize outside of FCS bottle with 70% ethanol prior to opening and adding to the media.
5. Add FCS. Refrigerate until needed. (Media should only be stored for 3-10 days in the refrigerator prior to use.)

*Water must be ice cold to dissolve some of the amino acids (Fig. 1B).

Antibody Production

Materials

1. Disposable, sterile pipettes
2. B cell line for producing MAb32B11
3. 50 ml tissue culture flasks (new, manufacture-sterile)
4. 500 ml tissue culture flasks (new, manufacture-sterile)
5. Incubator with temperature and gas regulation (ethanol cleaned)
6. Centrifuge tubes (new, manufacture-sterile)
7. Plastic dish with cover
8. 70% ethanol
9. RPMI media made above
10. DMSO

Methods

1. Using a disposable pipette, remove 10 ml of RPMI media and transfer to a 50 ml flask.
2. Remove cryo-vial containing the B cell line for producing MAb32B11 from liquid N₂ and place immediately into a plastic dish (with a cover) containing water at 37°C. Cover immediately. The vial will bounce around and could fly up and injure someone if not covered (Fig. 1).

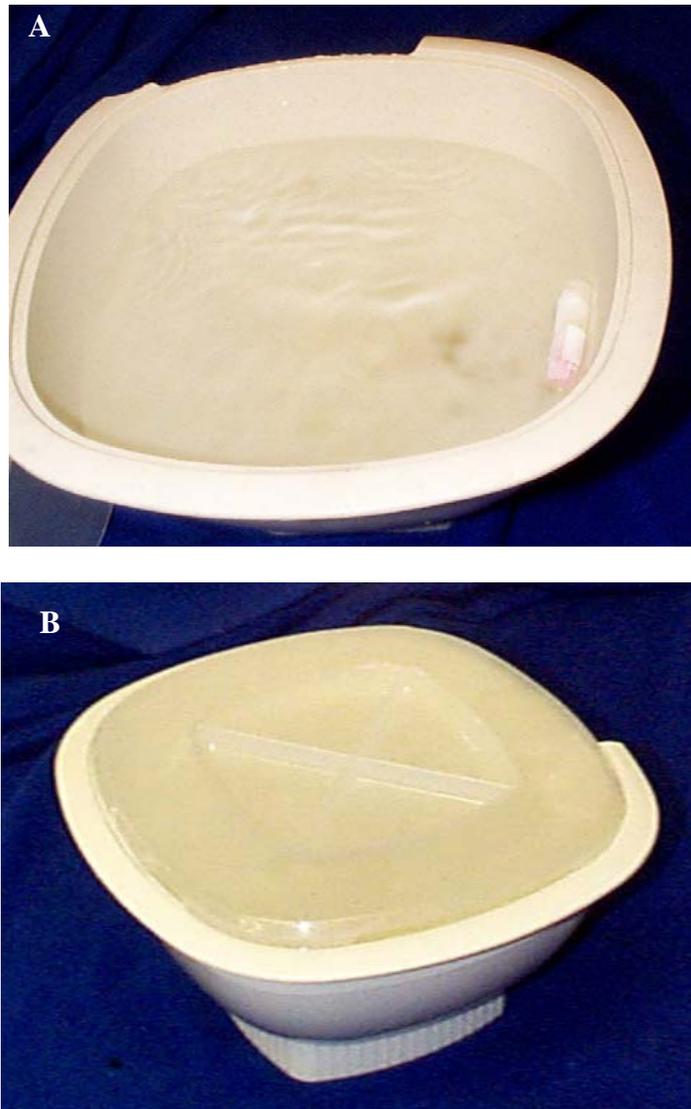


Figure 1. The cryo-vial is removed from liquid N₂ and immediately placed in water at 37°C (A). The vial will bounce around as it rises to room temperature. Cover to prevent injury (B).

3. Wait until vial quits bouncing around, remove cap and pour into 10 ml of RPMI media.
4. Place in incubator with slightly slanted shelves so flasks may be laid down (Fig. 2). Place a sterile tray in the bottom about 1/3 full of sterile (autoclaved) Milli-Q water (change water every 3 to 4 days). Flasks must have their caps loose or have caps with 0.22 µm filters to allow for gas exchange while maintaining sterile conditions. Incubator must be set at 37°C with 5% CO₂ (regulator at 10 psi).



Figure 2. Tissue culture flasks are placed at an angle in an incubator at 37°C with 5% CO₂ and sterile (autoclaved) Milli-Q water in a tray at the bottom.

5. Observe cells using an inverted microscope after 2 to 3 days. When the field of view is about 2/3 confluent (full), transfer 1 ml into a new flask with 10 ml of media (or all 10 ml into a larger 500 ml flask with 30-40 ml of media).
6. Add new media to the original flask until volume is 10-12 ml.
7. Check confluence every 2 to 3 days.
8. Keep transferring into new flasks and/or adding media (up to about 10-12 ml in the 50 ml flasks or 80-100 ml in the 500 ml flasks) until you have used all the media.
Note: Do not overfill flasks and do not allow media to be in the cap.
Note: If you are going to store some cells for future antibody production, leave about 5 to 10 ml of media and treat with 10% DMSO.
9. Let cells overgrow and die, media will change color slightly and become cloudy on the bottom.

10. After cells have died, transfer to sterile (new) centrifuge tubes and centrifuge at $3000 \times g$ for 5 min.
11. Pour supernatant into sterile (new) 50 ml containers. Centrifuge tubes or flasks work the best, because you can dispense easily from these tubes. Store in refrigerator.
12. Dispose of pellet containing cells.
13. Test centrifuged solution against a standard curve in an ELISA plate using a 1:6 antibody solution:PBS dilution factor to determine if this solution is strong enough to get good OD values for the standard.
14. If OD values are low, use a serial dilution with a higher dilution factor to determine the antibody dilution factor appropriate for your antibody solution.
15. To prevent contamination, antibody from the 50 ml container in step 9 may be dispensed into small increments (enough for one ELISA plate). This eliminates the need to open and pipette from a large amount of antibody (i.e. 50 mls) several times.

Storing cell line

Materials

Centrifuge tubes (new, manufacture-sterile), screw-capped 15 ml

RPMI media with DMSO (10%)

Nalgene Cryo 1°C freezing container

Isopropanol

Cryovials (1.5 mL)

Methods

1. Take a sterile, disposable pipette and mix the media in a 50 ml flask with overgrown cells from above procedure by moving the media up and down several times in the pipette and rinsing off the back of the flask to remove cells that have attached to the flask.
2. Transfer all the media into 15 ml centrifuge tubes.
3. Fill tubes to equal volumes to balance and centrifuge at $1500 \times g$ for 5 min. Pipet off supernatant.
4. Add enough (about 1-2 mls) RPMI media plus DMSO to resuspend the cells.
5. Resuspend the cells and transfer suspension to the cryovials.
6. Place cryovials in Nalgene Cryo 1°C freezing container*. Place immediately in -80°C freezer. (Cells will freeze slowly to prevent death when transferred to liquid N_2 .)
7. After 12-16 hr, rapidly transfer cryo-vials from freezing container to cryo-canes, and place in a dewar above the liquid phase and in the vapor phase of liquid N_2 or in a cryo-freezer. Do not allow media in vials to thaw.

*Change the isopropanol in the container after no more than 5 freezes to keep the alcohol concentration at 100%.