Different application of cell line cultures

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I. Standard production using your hybridoma in a fetal bovine serum (FBS) supplemented medium.

- This is the most basic production system whereby your hybridoma is grown in FBS supplemented cell culture medium to the volume requested. The maximum cell density possible is attained, the cells are allowed to produce antibody in the medium until approximately 80% of the cell population becomes non-viable, and the medium containing the antibody is collected. Cells are typically grown in standard spinner cultures for this technique.

II. Standard production using your hybridoma in a serum-free cell culture medium.

- Production is as above except the hybridoma cells are weaned to serum-free medium prior to production.
- This may be important to investigators concerned with subsequent purification as the absence of extraneous serum proteins makes purification easier. Time is added (two to three weeks) to the delivery time due to the weaning process.

III. Production using Hollow-fiber Bioreactor / Special membrane design Integra flasks.

- This system is recommended for investigators requiring relatively large amounts of antibody at medium to high concentrations.
- Your hybridoma is weaned to serum-free medium and loaded into a hollow-fiber bioreactor or a new stationary system by Integra. Cells are grown in the extra-capillary space of the cartridge or in a special membrane area in the Integra flasks to very high densities. Typically, seven milliliters of medium containing the antibody is collected every two to three days over a time period necessary to obtain the total required amount of antibody. The antibody isotype is monitored during weaning and production.

Extraction of protein from the cell line for Western Blotting:

- Tissue culture cell line was in T-25 tissue culture flasks were treated with the desired treatment for suitable time.
- Remove the culture media. The following step should be performed on ice or at 4ºC.
- Cells were then washed twice by ice -cold PBS.
- Add ice cold RIPA buffer (100µL/107 cells).
- Scraped in lysing buffer off the dish or flask with a rubber policeman.

Extraction of protein from the cell line for Western Blotting:

- Transfer the cell suspention into a centrifuge tube.
- Sonicated for 2 min or Gently rock the suspention on either a rocker or an orbital shaker in a cold room for 1 hour to lyse cells.
- Centrifuge the lysate at (13000 x g for 20 min) in precold centrifuge and Immediately transfer the supernatant to a fresh centrifuge tube and discard the pellet and store at -20ºC .
- Determined the protein concentration of the lysates.
- Samples (30µg protein) were loaded on a 10% SDS-polyacrylamide gel and subjected to electrophoresis (60 mA, 1h).

Extraction of protein from the cell line for Western Blotting:

- Gels were transblotted on nitrocellulose membranes. Ponceau staining assessed blotting efficiency and verified that, in each experiment, the total protein load had been similar for each lane.
- Membranes were then washed with TBST at room temperature. Membranes were blocked in blocking buffer for one hour.
- Membranes were then incubated with primary antibodies (1:1000) against Actin in blocking buffer at 4°C overnight.

Extraction of protein from the cell line for Western Blotting:

- Membranes were washed 3 times with TBST buffer for 15 min and incubated with secondary antibody, anti-goat and anti-biotin (1:2000), at 25°C for 2 hours
- Then washed with TBST 3 times.
- The membranes were exposed to a mixture of peroxide and luminol reagent and the chemiluminesence quantified using a phosphoimager