Monoclonal Antibody Development

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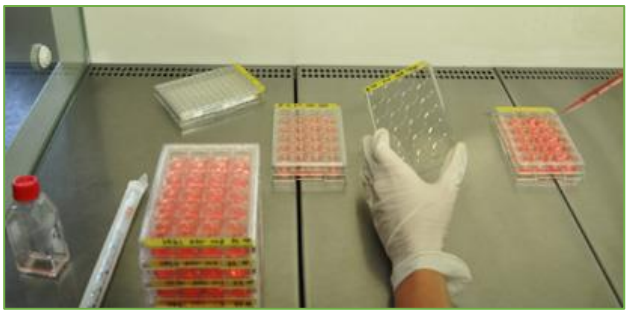
Introduction



Monoclonal antibodies are produced from one B-cell. Every antibody is identical as they derive from one clone. Usually they only recognize one epitope. The development of monoclonal antibodies is more time consuming in comparison with polyclonal antibodies. The background is usually lower, which lead to a clear signal in your applications. In addition, you can produce a limitless amount of your antibodies as you receive the immortal antibody producing clone.

Method

Step 1: Immunization

We immunize 4 BALB/c mice or 3 black hooded rats with different antigen concentrations to have a broad spectrum of immune responses. We perform ELISA titer determinations to detect the animal with the best response. The mouse with the best titer is used for the next steps.

Step 2: Isolation of spleen cells

The accurate isolation of the spleen cells including the antibody producing B-cells is important to continue the monoclonal antibody development. The spleen cells can directly be used for fusion or they can be stored in liquid nitrogen.

Step 3: Fusion

The isolated B-cells are fused with myeloma cells. Antibody producing hybridoma cells with endless growth are generated. By cultivating the cells with HAT medium, only the hybridoma cells survive.

Step 4: Screening and Cultivation

We grow all fusion cells and screen for positive clones in cell culture plates (alternative screening methods are available). Positive clones are grown in flasks and screened for stability and antigen specificity. We can screen against multiple antigens. The best clones are isolated and cryoconserved.

Step 5: Subcloning

After screening the clone may contain multiple antibody producing clones. To ensure that the antibody clone is monoclonal (all antibodies come from one B-cell clone) we perform limited dilution. The cells are diluted that only one cell grow in one well. A subsequent screening ensures that the subclone with the best antigen recognition is used for production & purification.

Production & Purification

The production is performed in serum free medium or in medium with FBS. Purification is performed with ProteinA/G/L or chromatographic methods. The production can be performed with IgG, IgM or other subclasses.