Monoclonal Antibody Development

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

The process of developing monoclonal antibodies with the hybridoma technology involves five steps: immunization, cell fusion, selection, screening, and cloning. Monoclonal antibodies are developed from B cells (usually from isolated spleen cells) that produce antibodies. They target specific proteins that induce an immune response. The initial monoclonal antibodies are created by fusing spleen cells from an immunized mouse with myeloma cells (malignant self-perpetuating antibody producing cells). Subsequent selection of hybridoma that show the best response against the target protein are used to produce the antibodies.

For this reason, every antibody is identical as they derive from one clone and they usually only recognize one epitope. Therefore, 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. In comparison with antigen specific polyclonal antibodies, the reaction might be lower as only one epitope from the same antigen is detected.

- 2 - Method

Step 1: Immunization

The immunization of 4 BALB/c mice or 3 black hooded rats with different antigen concentrations leads to a broad range of immune responses. The titer is determined by ELISA to get the animal with the best response against the antigen. The best responder 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 be used directly for fusion. When a direct fusion is not possible the cells can be cryo-conserved and stored at -80°C or lower.

Step 3: Fusion

The fusion of the isolated B-cells is done with myeloma cells. This results in antibody producing hybridoma cells with endless growth. At this step the cells are cultivated with a medium containing HAT (Hypoxanthin, Aminopterin, Thymidin). Spleen cells that did not build hybridoma will decay over time automatically. However, myeloma cells with an eternal life would live longer. For this reason the Aminopterin prevents the de novo synthesis of nucleic acids. The hybridoma have an alternative way to synthesize the amino

acids using the Hypoxanthin and Thymidin. Once the hybridoma decayed, the medium is changed to HT (Hypoxanthin, Thymidin) medium to recover the hybridoma. Then the medium can be exchanged to one without HT.

Step 4: Screening and Cultivation

All hybridoma are cultured in cell culture plates. The screening is done by ELISA with your target antigen (alternative screening methods i. e. with a negative screening are available). Positive clones that show activity against the target are upscaled and grown in cell culture flasks. At this step the hybridoma are screened and selected for stability and antigen specificity. Screening against multiple antigens is possible. The best hybridoma are isolated and cryo-conserved.

To determine which hybridoma produces the best antibodies in your applications, a small production and purification from the best hybridoma is performed. Our customers will receive an aliquot of the purified antibodies to use them in their applications. Please note that at this step the antibodies may not be fully monoclonal and that you might need a larger concentration.

Step 5: Subcloning

After screening the hybridoma may contain multiple antibody producing clones. To ensure that the antibody clone is monoclonal (all antibodies are produced from one B-cell clone) we perform a limited dilution. The cells are diluted in a way that only one cell grow in each 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.