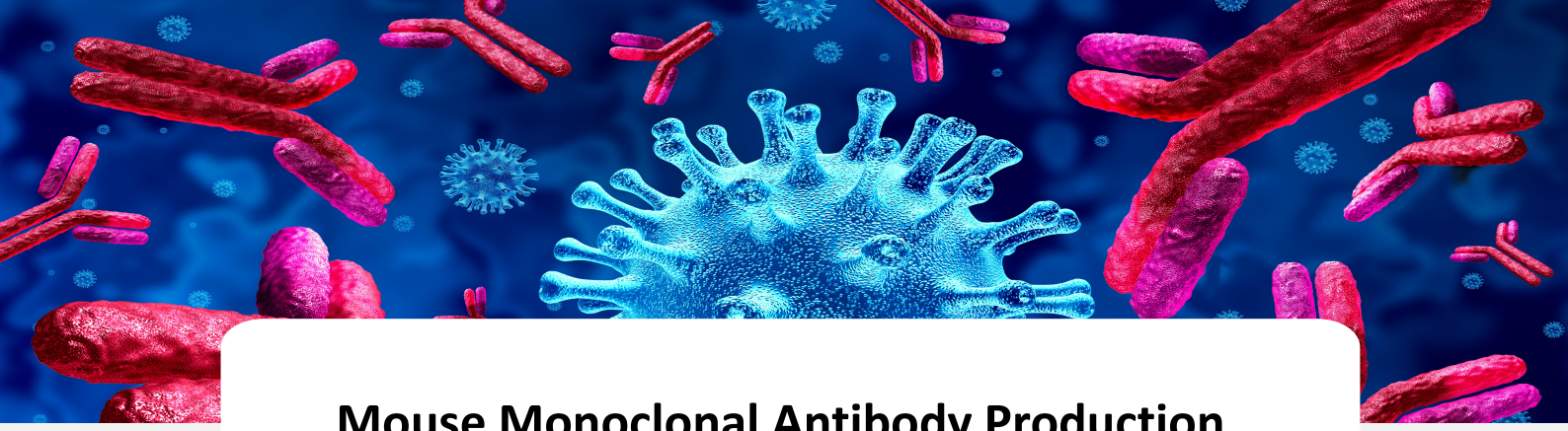

Mouse Monoclonal Antibody Production

Alpha Lifetech

1. Production of mouse monoclonal antibody

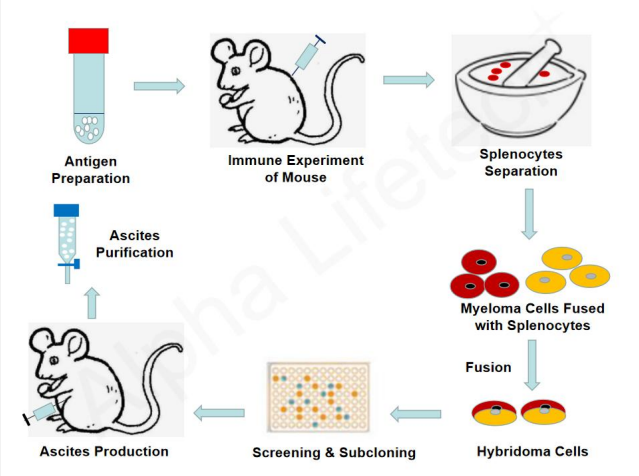

Fig1 Production of mouse monoclonal antibody

The steps of mouse monoclonal antibody production are as follows:

1) Antigen preparation

A. Peptide design & synthesis: peptide design, peptide synthesis, conjugation.
B. Small molecule design & conjugation: Small molecule modification, conjugation.
C. Protein preparation: cDNA synthesis, vector construction, protein expression & purification.
D. Virus particles (Inactivated or attenuated).

2) Purification & detection

A. Protein A/G purification
B. Antigen specific affinity purification

3) Immunization

A. Immunized 4-5 mice (Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were injected subcutaneously on day 14, 21, 38, 52, 68.)
B. Serum ELISA detection: ELISA titer>10^5 (Protein antigen, virus particles), ELISA titer>10^4 (Peptides or other molecules)
C. Fusion & Screening: Myeloma cells fused with splenocytes
D. Subcloning
E. Ascites production (injection on 5 mice)

2. Q&As for Hybridoma cell line

Q1: How to choose the right fusion hybridoma?

A1:

During the fusion process, 10-15 96-well microtiter plates are seeded with the fusion hybrid mixture. Each plate is fed HAT-IMDM selection media and maintained in a carbon dioxide incubator at 37 degrees Celsius. 9 - 14 days after the fusion, hybrid supernatants are tested for the presence of the specific antibody of interest.

Fusion of plasma cells with their myeloma counterparts is not 100% effective. Even under optimal conditions and the most effective stimulation, cell fusion still produces a mixture of unconfluent and confluent cells that need to be separated. To improve the efficiency of the selection process, myeloma cells used for fusion lack HGPRT, a key enzyme in the nucleotide salvage pathway. The mixture was then cultured in HAT medium, where only cells with the HGPRT enzyme, hybridomas that inherited HGPRT from plasma cells, were viable.

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Q2: How to screen hybridoma cell lines for antibody activity?

A2:

Evaluation of hybrids is a critical step. Generally, many companies provide hybridoma, clone and subclone supernate screening by enzyme-linked immunosorbent assay (ELISA), Western blot assay and fluorescence-activated cell sorting (FACS). To reduce costs, scientists may choose to perform all screening of supernates in their own lab.

Hybridoma supernates to be tested may range from 500 to 1,440 samples and will be ready to test by days 9- 14. They could arise over a three- to five-day period or may all be ready on the same day, so it's important for the client lab to be prepared weeks in advance with working specific secondary screening assays of choice.

Q3: Why do positive hybridomas need to be subcloned?

A3:

After positive Wells were identified during the initial ELISA screening procedure, hybridomas were transferred to a larger volume, i.e., 24-well plates, in preparation for the subcloning procedure. Subcloning of hybridomas is usually performed by a limited dilution method to ensure the isolation of stable monoclones. The method involves diluting hybridoma cultures and dispersing them into 96-well plates to achieve monoclonality (one cell per well). To reduce the risk of developing mixed hybridoma populations, at least two limited dilutions should be

Q4: What are the benefits and limitations of using hybridoma technology for antibody discovery?

A4:

Benefits: Hybrid cell lines have been praised for their ability to produce antibodies with high affinity, stability, and specificity in the most cost-effective manner.

Limitations: In contrast to in vitro antibody generation techniques, the development of hybridomas and their corresponding myeloma fusion partners requires significant time.