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SCAN-BRIEF

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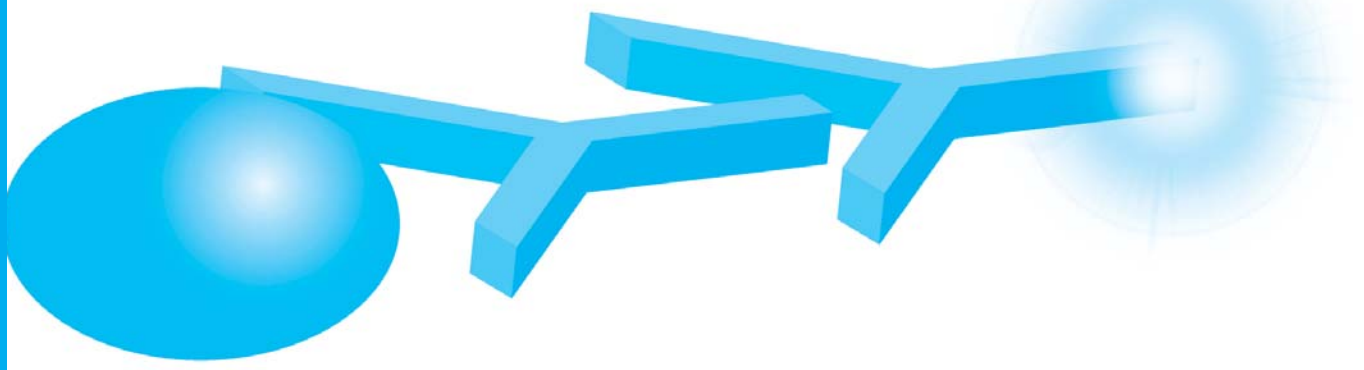
SCANTIBODIES Laboratory, Inc.

Contract Monoclonal Antibody Production



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- Contract *In Vitro* (bioreactor) MAB production
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- How SLI documents the details of quality control and production

Contract Monoclonal Antibody Production

Introduction

Over the past ten years, Scantibodies has built up an efficient, cost effective system of manufacturing monoclonal antibodies on a contract basis. The following is a description of the overall system and procedures used when Scantibodies performs custom monoclonal antibody (MAB) production. There are two types of custom monoclonal antibody production, *in vivo* (typically in mice), and *in vitro* (using bioreactors). For cost, speed and scale up reasons, the *in vivo* type has been the most popular method of choice.

Custom *in vivo* monoclonal antibody production is accomplished using a wide variety of cell lines. At Scantibodies BALB/c mice are maintained in a breeding colony with a population of 100,000 mice. Other strains of mice (Swiss Webster, CAF-1, ICR, etc.) are also available for antibody production. Custom *in vitro* monoclonal antibody production is performed in different types of bioreactors.

There are three types of price plans available for custom MAB (Monoclonal Antibody) production. The price can be based on any one of the following:

1. Per volume ascites
2. Per milligram of antibody in ascites or supernatant media (non-purified form)
3. No Risk Plan (per milligram of antibody in purified form)

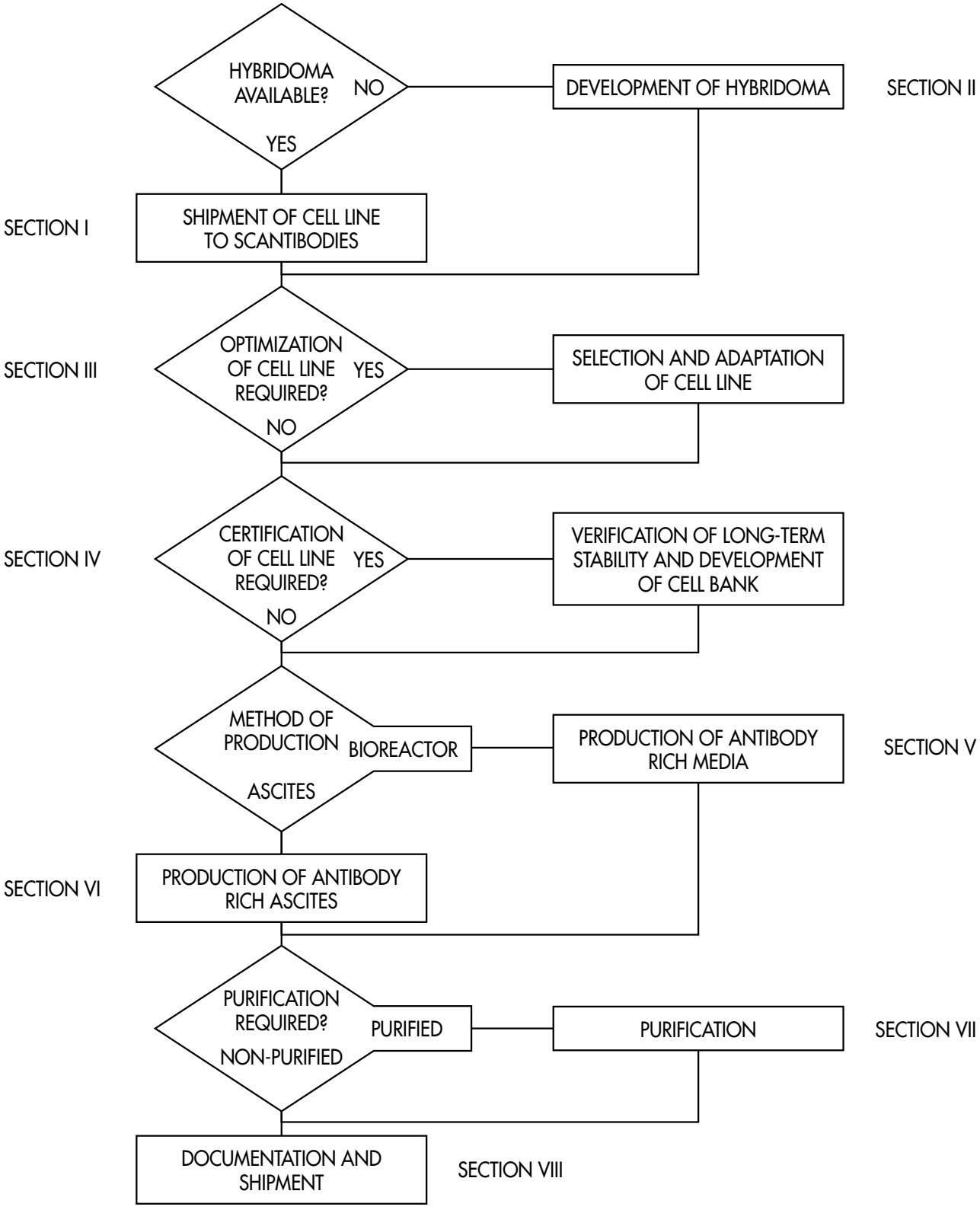
By purchasing the MAB on the basis of purified, functional antibody, risk to the customer is eliminated. When a price quote is based on forms other than purified MAB (i.e., number of mice or bioreactor, volume of ascites, etc.), the final MAB price is indeterminate. Limits in hybridoma antibody production and losses incurred during the purification process can account for dramatically increased cost.

MAB Production is a Multistep Process:

1. The first step in MAB production is hybridoma development. SLI (Scantibodies Laboratory, Inc.) offers hybridoma development service if there is no existing hybridoma available to the customer.
2. Alternatively, an existing hybridoma is shipped to SLI utilizing SLI shipping procedures. Careful shipment ensures the safety of the cell line.
3. SLI can offer cell line optimization for maximum MAB production.
4. Cell line drift, one of the greatest dangers encountered by a cell line, can be prevented with close monitoring and cell line certification.
5. Custom MAB production is offered in ascites and, as an alternate method to *in vivo* production, SLI can propagate a large number of antibody producing cells *in vitro* using bioreactors.
6. A customized, multistep purification process is performed to meet MAB specifications.
7. Shipments of MAB's are accompanied by a detailed certificate of analysis.

All procedures are discussed in detail in sections indicated on the flow chart.

Monoclonal Antibody Production



SECTION I

SLI Facilitates All Shipping Procedures to Ensure the Safety of the Cell Line

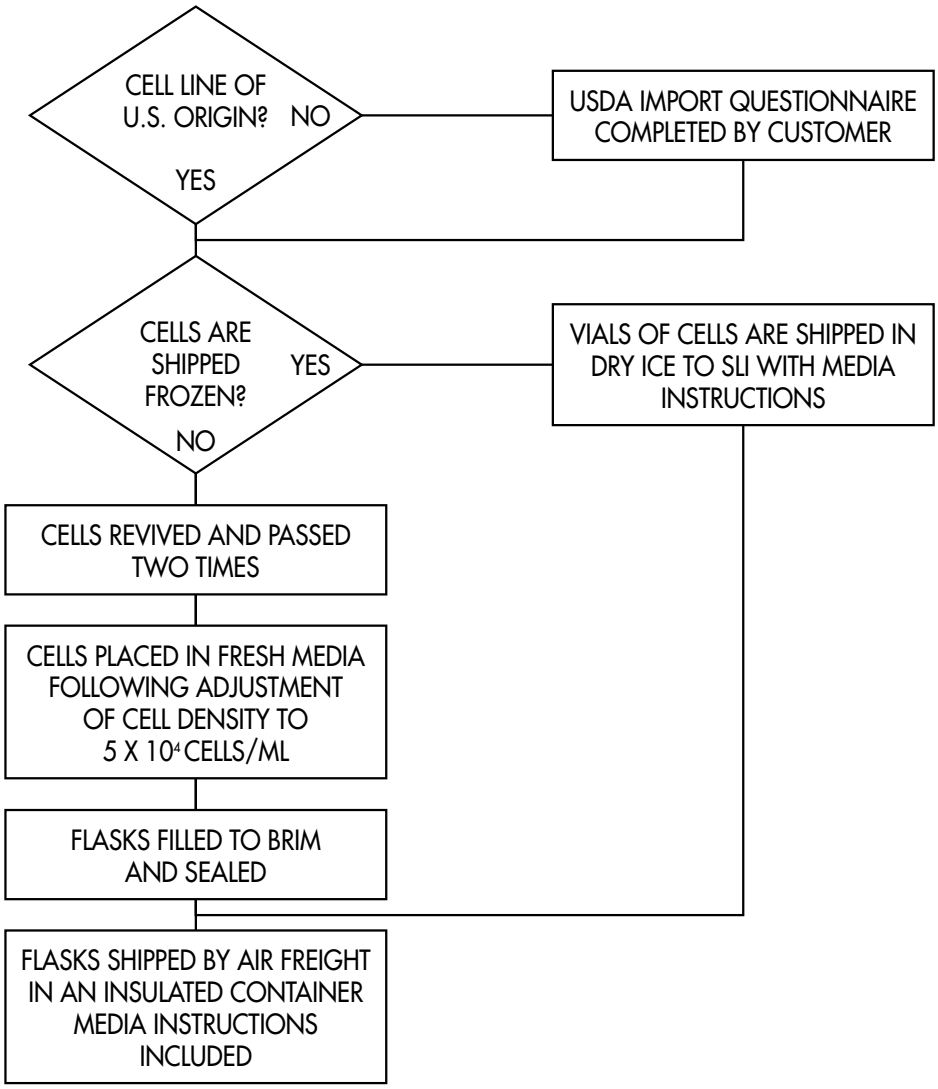
For shipments from outside the U.S.A., SLI provides a questionnaire to the customer so that SLI may receive the shipment of cells.

If cells are to be shipped non-frozen, the customer must revive the cells and check for viability. The cells are then passed two times and placed in fresh media following cell density adjustment to $5x10^4$ cells/mL.

Flasks are filled to the brim, sealed and shipped to SLI by air freight in an insulated container to maintain ambient temperature.

All shipments of cells must include media instructions.

Shipment of Cell Line to Scantibodies



SECTION II

Development of Hybridoma Cell Lines

Because antibody requirements differ according to assay specifications, there can be no single procedure for hybridoma development to satisfy the requirements of all assays. **SLI develops a customized strategy to generate the best hybridoma possible for each antibody produced.**

The SLI staff develops monoclonal antibodies directed against many immunogens (antigens) including peptides, large protein molecules, viruses, bacteria and mammalian cell surface antigens.

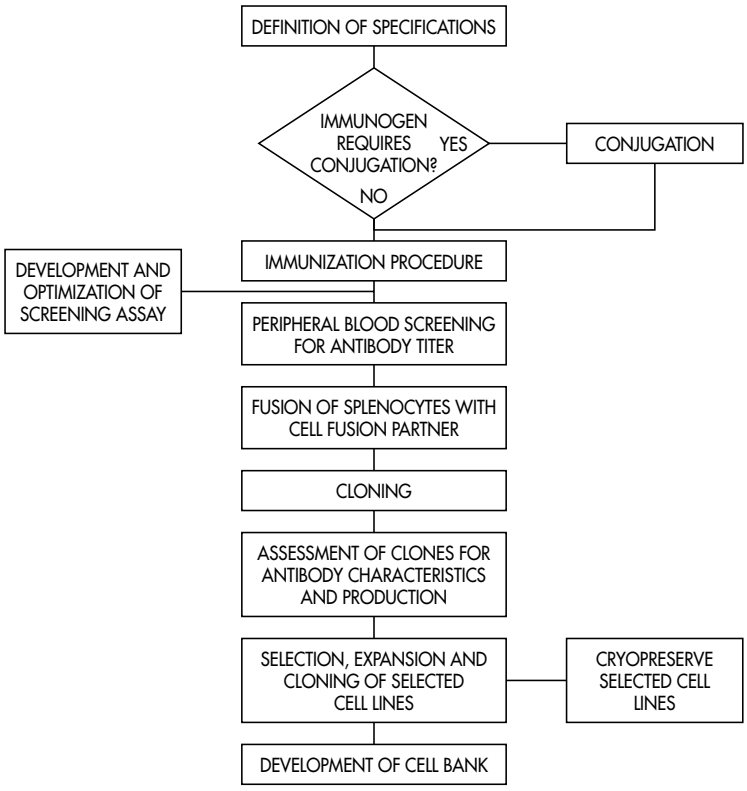
The development of a hybridoma cell line occurs in a series of phases:

Following the determination of MAB specifications and conjugation of the immunogen, if required, mice are immunized at 1 week intervals with an appropriate adjuvant. The first test bleeds are taken 1-2 weeks following the initial immunization and the peripheral blood sera are titered with an optimized antibody screening assay. Modified to satisfy specified requirements, this screening can consist of ELISA, radioimmunoassay and immunoagglutination. An antibody-secreting cell line is produced by the polyethylene glycol (PEG) mediated fusion of a B cell from the lymphoid tissue of the immunized animal and a cell from a plasmacytoma (myeloma) cell line. In order for the hybridoma to maintain characteristics of each parent cell, B cells and myeloma cells used must be of similar lineage and stage of development. Plasmacytoma cell lines employed possess a deficiency in the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT), rendering them unable to synthesize DNA when cultured in media containing hypoxanthine, aminopterin, and thymidine (HAT medium). The lymphocyte parent cell complements this deficiency during the fusion process. Lymphocytes are unable to survive long term in culture. This ensures that only hybridoma cells (resulting from the fusion of the lymphocyte and plasmacytoma cell) are selected for growth. The resultant hybridoma retains the capability of one parent cell (lymphocyte) to secrete the antibody and the continuous growth characteristics of the other parent cell (plasmacytoma). The hybridomas are then screened with an immunoassay and selected for optimal antibody characteristics and production.

Hybridomas selected for further development are cloned with the limiting dilution method in 96-well microtiter plates. With the limiting dilution method, cells are easily monitored throughout the cloning process.

Monoclonality is confirmed and the clones are assessed for antibody activity. Selected hybridomas are grown until the cultures are large enough to screen for antibody production (approximately 2-3 weeks). Positive hybridomas are expanded, subcloned, and cryopreserved to form a cell bank.

Subcloning of a cell line assures monoclonality.



SECTION III

Cell Line Optimization

The critical optimization procedure developed at SLI dramatically increases MAB production. Small scale lots are manufactured to confirm the hybridoma's productivity.

Bioreactor Production

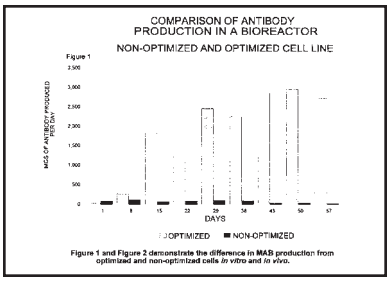
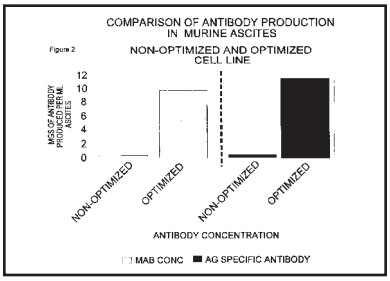


Figure 1 and Figure 2 demonstrate the difference in MAB production from optimized and non-optimized cells *In vitro* and *In vivo*.

Optimal conditions for cell growth and MAB production are determined, including the appropriate media for the promotion of optimal growth, the best bioreactor, the volume of the inoculum and the appropriate bioreactor operating parameters for maximum MAB production.

A mass culture or a bioreactor is inoculated with the selected cell line. The media is harvested according to established procedures.

Ascites Production



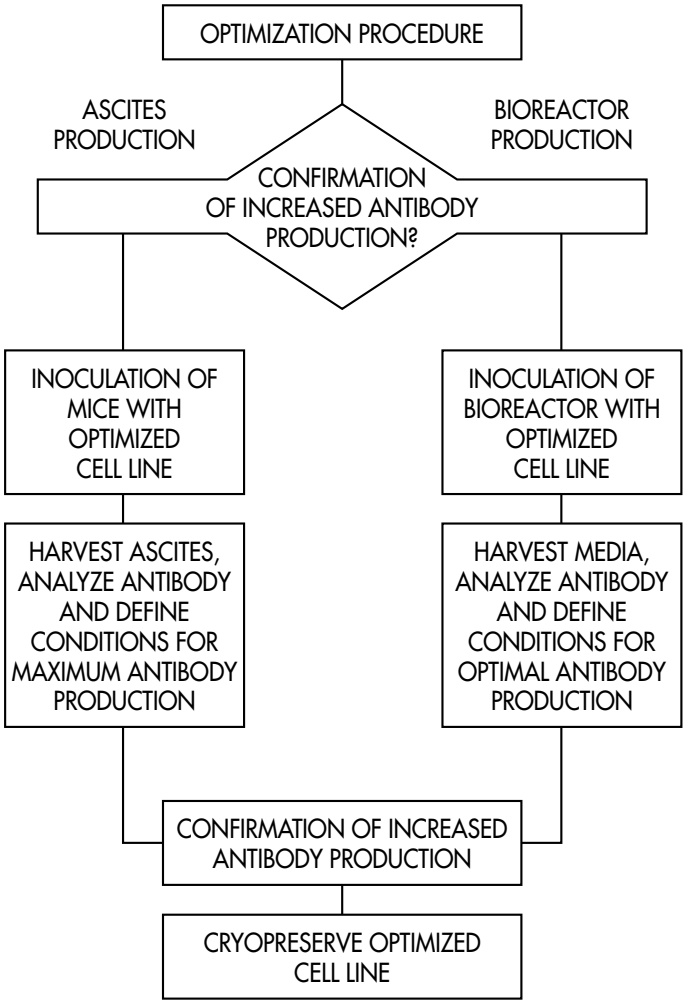
Optimal conditions for maximum antibody production are determined, including the pristane dose, the time interval between pristane priming and inoculation, the density of inoculum, and the age, strain and sex of mice appropriate for the MAB production.

Mice are then inoculated with 10^6-10^7 cells/mL. Ascites is harvested 2-3 weeks following inoculation.

The ascitic fluid contains 10-50 times the antibody concentration of the supernatant of hybridoma cells grown in culture.

Cell lines are selected for maximum antibody production, activity and specificity using HPLC and immunoassay techniques.

To ensure the security of the selected cell line, aliquots are cryopreserved in vials located in two SLI storage sites.



SECTION IV

Prevention of Cell Line Drift

A cell line is comprised of a large population of cells. Over time, mutations occur within the population. Some mutations give rise to virulent, non-antibody producing cells which threaten the security of the cell line if they are permitted to grow. This condition, which is representative of one of the greatest dangers encountered by a hybridoma cell line, is referred to as cell line drift. Continuous monitoring and cell line certification is therefore essential.

At SLI, large seed stocks can be maintained for each hybridoma cell line where they are closely monitored for hybridoma viability, the absence of microbes, antibody expression and isotype uniformity.

After the cell line is revived, percent viability is determined with a trypan blue contrast assay.

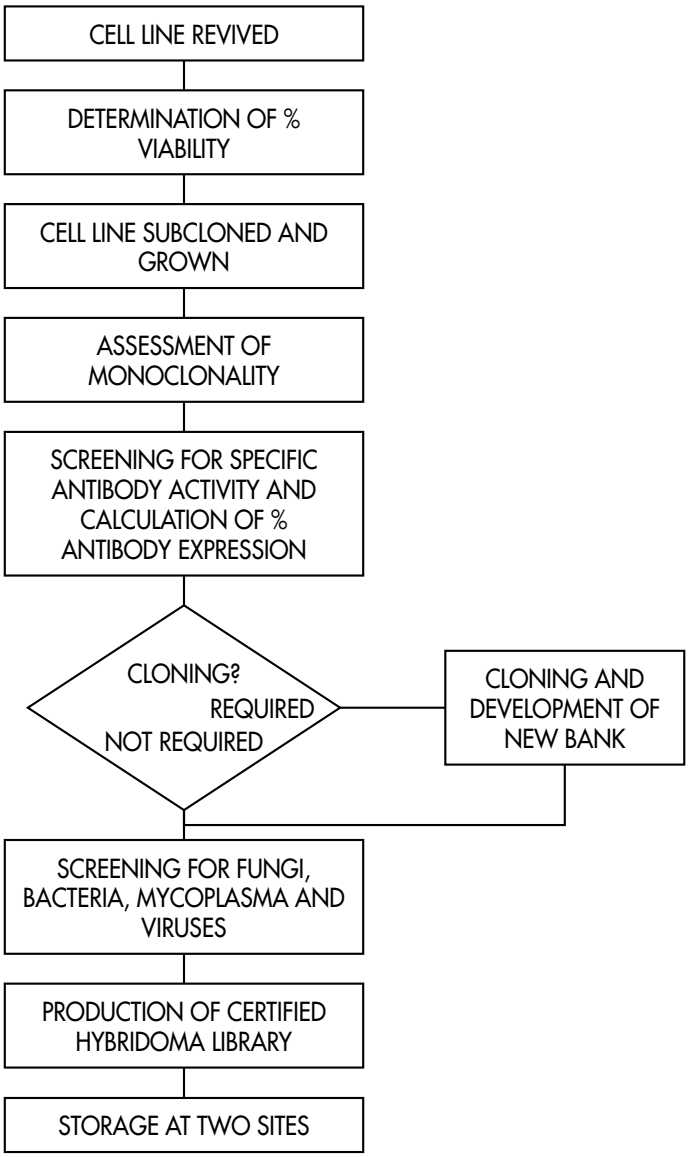
The cell line is then subcloned in order to assess monoclonality.

Antibody activity is assessed with an immunoassay and the percent of specific antibody expression is calculated.

If necessary, the cell line is subcloned with the limiting dilution method to reinstate monoclonality preceding the development of a new cell bank.

All cell lines are screened for fungi, mold, microbial growth, mycoplasma and viruses prior to the development of a certified hybridoma bank. Aliquots are stored in multiple SLI sites to ensure the security of the cell line.

Certification



SECTION V

Contract *In Vitro* (Bioreactor) MAB Production

Due to the absence of endogenous mouse IgG and certain serum contaminants encountered during *in vivo* MAB production, the purification process is simplified for bioreactor produced MAB.

Immunological functionality for bioreactor produced MAB can be up to 10% greater than the immunological functionality for ascites produced MAB.

Prior to the production run, selected bioreactors are qualified to meet pre process specifications. Qualified bioreactors are then inoculated with selected cells (generally 10^8 cells per bioreactor).

Operation of the bioreactor is closely monitored to ensure adherence to defined production parameters. Media containing additives required for optimal cell growth is used. Cell growth and antibody production are assessed with the periodic measurement of glucose uptake, pH, lactate concentration, antibody yield, temperature, gas exchange, etc.

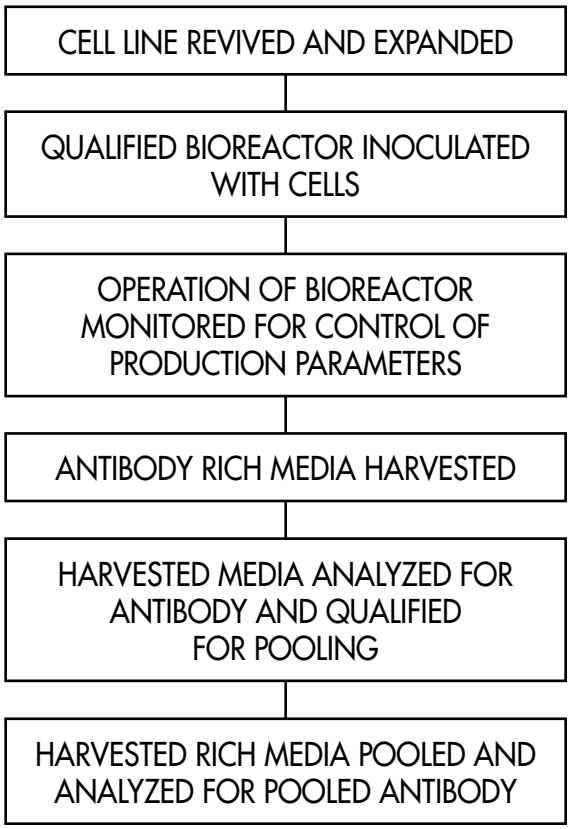
The supernatant media begins to be harvested approximately 1-3 weeks following hybridoma inoculation. The harvested supernatant media is analyzed for antibody by physico-chemical analysis This analytical data provides the basis for sub lot harvest qualification for eventual main lot pooling.

All points of the SLI Quality System are strictly adhered to for *in vitro* MAB production procedures.

Following the definition of MAB requirements and release specifications, a design for production is developed. Process specifications including detailed procedures, technical requirements of production facilities and equipment as well as requisites for the production staff are determined. All members of the production staff are rigorously trained, certified, periodically tested and appropriately retrained in accordance with ISO and GMP requirements.

Specifications for raw materials are defined. All raw materials used in the bioreactor production must undergo qualification procedures. Vendors from which materials are being obtained are qualified in compliance with SLI Quality System Standards. Upon arrival at SLI, all raw materials are quarantined until they are tested against set specifications.

Bioreactor Production



SECTION VI

Contract *In Vivo* (Mouse Ascites) MAB Production

The breeding colony at SLI maintains 100,000 BALB/c mice. The colony is maintained under closely controlled conditions by animal care experts who take great interest in the welfare of the animals as well as MAB production procedures.

A breeding stock and a working stock of mice are maintained. Mice are transferred to the working stock once they have reached the maturity level required for ascites production (approximately 6 weeks).

The ventilation systems in the mouse facilities guarantees complete air replacement over 10 times an hour. This maintains constant temperature and helps prevent viral or other microbial contamination.

Analogous with *in vitro* production methods, all points of the SLI Quality System are strictly adhered to for *in vivo* MAB production procedures.

The first step for *in vivo* MAB production is to revive the cell line.

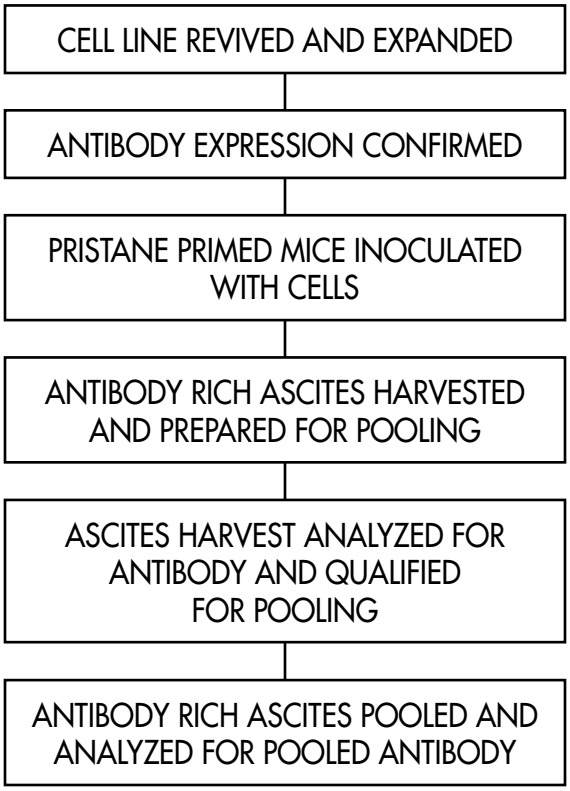
The mice are then pristane primed and aged to promote tumor formation and enhance antibody production.

The mice are inoculated with the hybridoma cells.

Approximately 2-3 weeks following inoculation, the ascites is harvested, prepared and qualified by physico-chemical analysis for main lot pooling.

In order to prevent immunological rejection of the hybridoma by the inoculated mouse, only syngenetic animals are used for ascites production. A syngenetic animal is genetically identical, at the major histocompatibility loci, to the animal from which the hybridoma was derived.

Ascites Production



SECTION VII

SLI Offers Multiple Purification Techniques for Monoclonal Antibodies

Ion Exchange Chromatography (DEAE)

DEAE separates proteins on the basis of net charge. The DEAE column support contains ions which can be competed out (exchanged) with ionic solutes in the mobile phase. This method offers a high resolving power and is capable of separating a variety of immunoglobulin types (IgG, IgM, IgD, IgA).

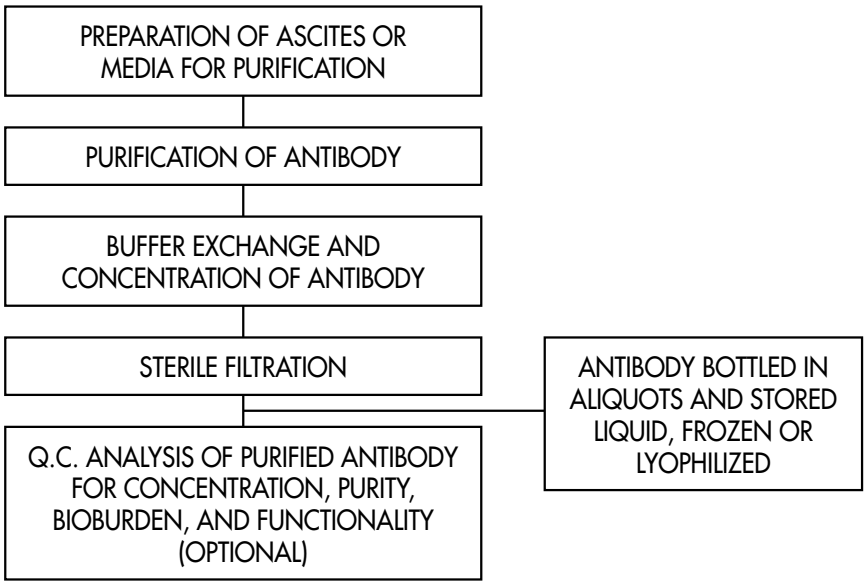
Protein A/G Purification

With the protein A/G method a mixed subclass IgG preparation can be separated out by eluting a protein A column in a single step. This method is highly specific and can be used with almost all isotypes of IgG's.

Alternative and/or Supplemental Purification Methods are Also Available

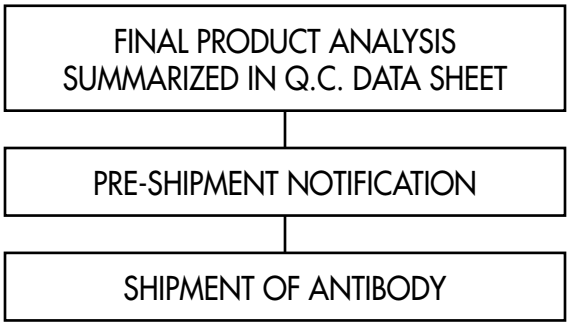
Following the purification process, the ascites or media undergoes buffer exchange, antibody concentration and sterile filtration through a 0.2 micron filter.

The purified antibody is then bottled and stored in aliquots in liquid, frozen or lyophilized form depending on the product specifications. A Q.C. analysis assessing the purified antibody concentration, purity, bioburden and functionality (if specified) is performed.



SECTION VIII

Documentation and Shipment



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