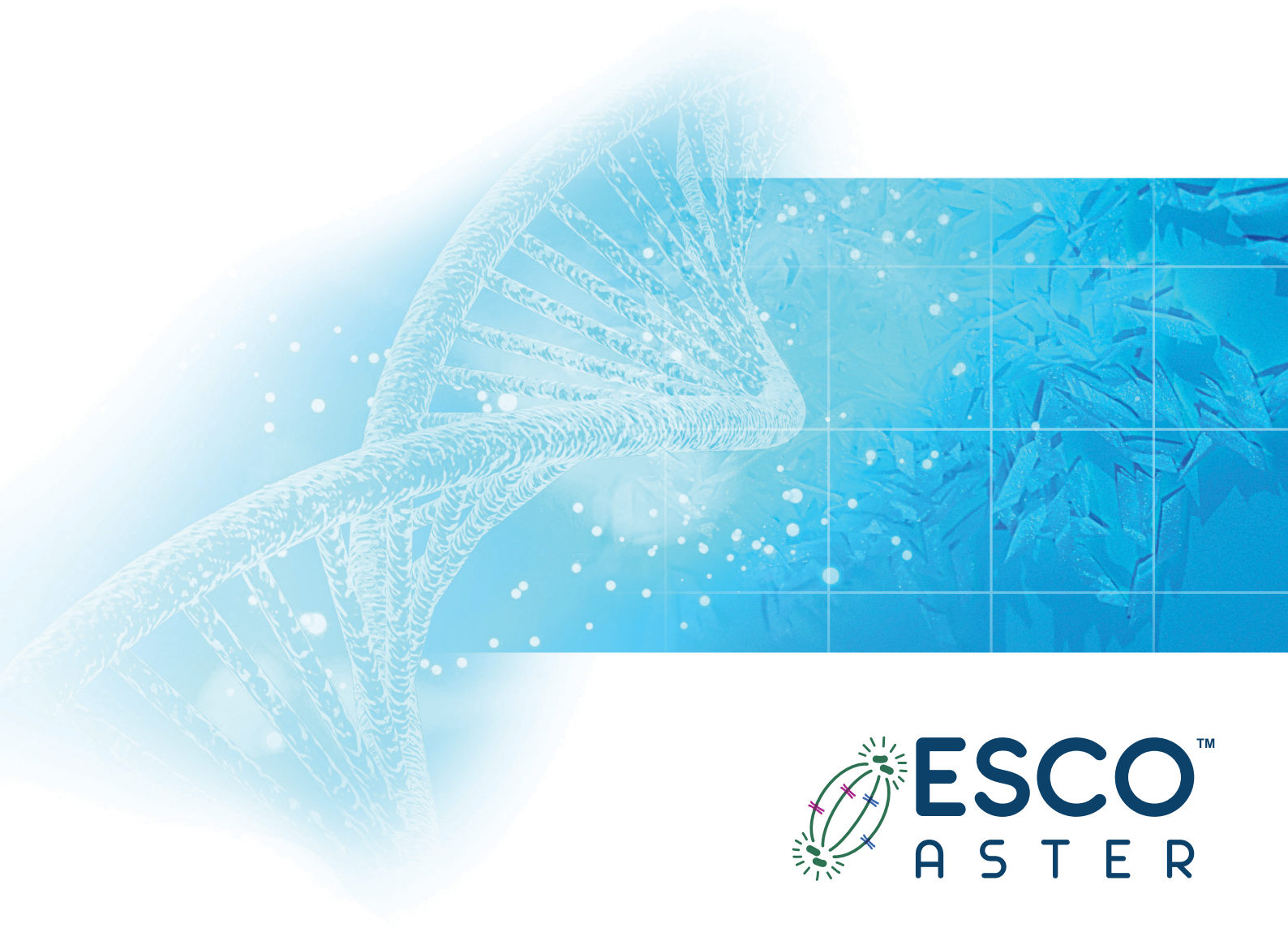
Application Note

Monoclonal Antibody Production



ESCO™ ASTER

High-Titre Monoclonal Antibody Production Using TideMotion™ Bioreactor

Background and **Introduction**

Hybridoma lines are generally created by the HAT (hypoxanthine-aminopterin-thymidine) selection method. These aim to select out unfused spleen/ myeloma cells to result in a culture enriched in hybridoma cells. Hybridomas can produce monoclonal antibodies (mAbs) with high titre only if induced under high-stress situation. There is no generalized and optimized cell culture protocol for hybridoma cultures because optimum growth conditions for every individual clone will vary.

A 2D system, e.g. T-175 flasks, or any flask-based cell culture system, is initially used to grow and expand the cells using cell-growth optimal media followed by cell-induction medium. This technique can be applied in combination with partial serum starvation to acclimatise the hybridoma cells.

The purpose of this application note is to provide scientists with a generic condition for the first CelCradle optimization experiment. Key components and the design methodology deployed in single-use CelCradle-500AP bottle in perfusion mode will be highlighted. It is recommended for one to read this application note prior to conducting the first experimental run.

Setup and **Materials**

Device	Cell Line	Media	Seed
CelCradle-500AP	Hybridomas	Prepare 500 ml for CelCradle-500P bottle using IMDM containing10%FBS and $NaHCO_3$ + Glutamine Overfill and prepare 5.5L in a 5 L glass vessel using IMDI containing10%FBS and $NaHCO_3$ + Glutamine	$1x10^8$

Table 1: Materials required for performing Phase I – hybridomas cell expansion.

CelCradle Volume	TideMotion Parameters		Growth and Acclimation Period(s)
500 ml (initial batch)	**Day 0 to Day 4** Uprate: 2 mm/sec Uphold: 2 mins Downrate: 2 mm/sec Downhold: 0 sec	**Day 5 to Day 12** Uprate: 2 mm/sec Uphold: 2 mins Downrate: 2 mm/sec Downhold: 30 sec	***Adaption of serum levels**
Perfusion Bottle Volume	**Perfusion Parameters**		
5.5 L	**Pump 1** Volume: 1999 ml Cycles/ Day: 24 Schedule: 1111111		

Table 2: Overview of CelCradle stage growth and perfusion parameters for growth and acclimatisation periods.

The single-use CelCradle-500AP introduction to the gentle mixing of the TideMotion (under constant flow rate and improved oxygenation) hosts the co-existence of both suspension cells (~ approximately 50-70% population in media) and adherent cell cultures (~ 50%-30% population onto BioNOC™ II carriers).

Points to **Consider:**

1. It is suggested to isolate a single clone that is more adherent as well as being a high "producer" in nature and expand that clone for growth on BioNOC™ II prior to culture in CelCradle-500AP bottle.

2. It is critical to maintain the pH of the medium at range between 7.3 to 7.4 to ensure maximum attachment efficiency of cells on the carrier. Perfusion mode CelCradle-500AP bottle operates at higher concentrations and allows maximum control over culture conditions including nutrient, pH, gas and waste levels.

3. Specific growth rates, glucose and glutamine uptake and lactate and ammonia production rates are important parameters. If the glucose concentration in the reservoir (inlet) is below 1.0 g/L, it is advised to stop the culture and harvest the culture medium or change to another reservoir with fresh 5.5 L culture medium in order to continue culture. Glucose levels can be checked as regularly as an indicator of cell health.

4. As hybridoma cells are loosely adherent, it is best to handle the culture as gently as possible without disturbing the cells from the carriers.

5. Perform fed-batch cultivation using CelCradle-500AP bottle if you are developing serum-free substitute or by a weaning procedure (*adaption of serum levels) which can be modified in current setup by closing off the perfusion tubing valves after reaching high-cell density. This is considered an intermediate step in the expansion of cells before transferring them to a larger bioreactor.

This Application Note describes a general protocol for the first CelCradle optimization run to produce a hybridoma culture containing a mixture of monoclonal antibodies. Each hybridoma clone will exhibit different growth characteristics and production lifetimes, ranging from days to years.

Image 3: The CelCradle-500AP bottle is designed to fit into a standard CO_2 incubator (constant temperature and gas control).

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