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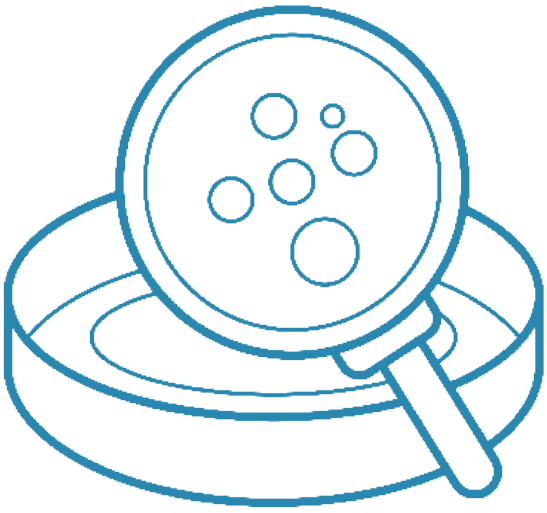
LeProlif™ Sphere

Product User Guide



The picture is for reference only, the actual item is the standard.

LeProlif™ Sphere User Guide

Introduction

LeProlif™ Sphere microcarrier is a kind of cell carriers for high yield culture of anchorage-dependent cells. It provides a suitable surface for the growth of animal cells and can be used in suspension culture system. It can also be used to increase cell production in monolayer cell culture containers (such as culture plates, culture dishes and culture bottles) and perfusion chambers.

Materials	Cross-linked dextran matrix with N, N diethylaminoethyl group (DEAE) for positively charged
Size	50~100μm(dry powder) ; 150~250μm (in solution)
Surface area	~4400 cm^2/g
Sterilization	Autoclaved sterilization（121 °C ,30min）
Method Concentration	3~5 g/L

Parameters

LeProlif™ Sphere meets specific design requirements in terms of technical parameters：

⦿ The optimized particle size and density support anchorage-dependent cells for better proliferation

⦿ The matrix has biological inertia and excellent transparency for observation of cell growth under the microscope

⦿ The positive charge is uniformly distributed on the surface with density between 1.4-1.6 mmol/g to ensures optimum adsorption of cells

Tests	Unit	LeProlif ™ Sphere typical value
Buoyant Density[1]	g/ml	1.030
Ionic capacity	mmol/g	1.47
Particle size[1,2]	d_{50}(μm) d_5-d_{95}(μm)	d_{50}:174 d_5-d_{95}:140-215
Approx. area (wet)[1,2]	cm^2/gdry powder	4400
pprox. no. microcarriers/g dry weight	个 /g	4.8 *10^6
Swelling factor [1,2]	ml/g	17
Heavy metals	μg/g	⩽ 5
Bioburden	CFU/g	<100

1.In 0.9% saline solution
2.May vary from batch to batch

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Preparation:

1. Weigh appropriate amount of microcarriers into the silicified container, hydrate microcarriers with Ca^{2+} and Mg^{2+} free PBS solution (50-100mL/g of microcarriers)

2. Hydrate and soak at room temperature for at least 1 hour, shake gently and mix well from time to time

3. Keep it still till microcarriers settle down

4. Decant the supernatant and add fresh $Ca^{2+}$and Mg^{2+} free PBS solution (30-50mL/g of microcarriers), autoclaved sterilization at 121°C for 30mins. (LeProlifTM Sphere with extreme stability can be repeatedly autoclaved for at least 5 times)

5. Cool down till room temperature and it can be stored at 4°C aseptic preservation

6. When the microcarriers have settled, discard PBS solution, add culture medium (20-50mL /g of microcarrier) and balance at 37 °C overnight

7. Discard supernatant, add fresh culture medium at room temperature and transfer them to the culture vessel; the PH of all solutions should be 7.0 to 7.4

Culture Vessels

LeProlif™ Sphere technically could be used in any type of cell culture vessels. However, it is most suitable to suspend microcarriers in the medium at a certain speed. Proper rotation speed can avoid excessive shear force providing a mixed and uniform culture system. There are many forms of stainless steel reactors, single-use bioreactors, glass reactors and rocking platforms that are suitable for using LeProlif™ Sphere microcarriers. LePure® provides different bioreactors like LePhinix® , LePhinix®CCS rocking platforms,glass bioreactors etc. to meet various cell culture requirements from customers.

Culture Procedure

The exact culture procedure and parameters depends on type of cells and culture vessels. The recommended filling density of microcarriers is 3-5 g/L with inoculation of 5×10^4 to 2×10^5 cells/ml, and the stirring speed is from 20 to 60 rpm, depending on different culture systems. In case of perfusion culture, the filling density can be as high as 20g/L.

The following is the process of suspending the microcarriers for cell cultivation in a 250mL double-arm rotary flask in the laboratory when testing the LeProlif™ Sphere microcarriers for the first time（the volume and inoculation density can be adjusted appropriately according to different cultivation volumes and containers）

1. Add 0.3g of LeProlif™ Sphere microcarrier into 90ml culture medium
2. Add 10ml solution containing 10^7 cells, and the constant volume is 100ml
3. The stirring paddle rotates slowly and the temperature is maintained at 37 °C
4. Intermittent stirring mode is adopted to make cells evenly adhere to the surface of microcarrier; Continuous stirring can be started when the cell adhesion rate reaches more than 90%; Adjust the rotation speed according to the cell adhesion to ensure the uniform distribution of cells and microcarriers
5. During this period, a small amount of samples can be taken to observe the cell adhesion or growth under the microscope, and the cell density and viability can also be monitored by the cell counting equipment; Through observation and test data, flexibly adjust rotation speed and optimize cultivation parameters
6. If manual perfusion operation is required, the filling amount of microcarriers (10-20g/L) can be increased to 1-2g at the initial stage; Stop stirring when changing the solution. After the microcarriers have settled completely, extract the supernatant and then add new medium.

Notice:
- Make necessary adjustments in parameters and process according to different types of cells.

Harvesting Cells

There are different methods to separate cells from microcarriers, among which proteolytic enzymes such as pancreatin are commonly used.

1. Remove the supernatant medium after microcarriers settled

2. Wash twice with PBS solution free of Ca2+and Mg2+and containing 0.02% (w/v) EDTA (50-100 ml/g of microcarrier)

3. After removing EDTA-PBS solution, add trypsin (30-50ml/g of microcarrier); Fully mix and incubate at 37 °C , slightly shake

4. 5-15 minutes later, observe under the microscope that the cells have fallen off the microcarriers, then immediately add serum-containing medium (20-30ml/g of microcarriers)

5. The detached cells can be harvested from the microcarriers by sedimentation at unit gravity(for routine harvesting) or by filtering through a 100um filter(for maximum recovery) and used fro the inoculation of a subsequent microcarriers culture

Notice:

- Make necessary adjustments in parameters and process according to different types of cells.

Quality Control

Each batch of LeProlif™ Sphere microcarrier is subject to strict quality control. It can only be released after passing the physical performance, biological safety and cell culture performance tests. The COA records the test data of each batch. For detailed indicators, see the COA certificate sent with the product.

Product Specification

LeProlif™ Sphere microcarriers are white dry powder and needs to be hydrated and sterilized before use.

The following are the different specifications of the product：

SPEC.	25g	100g	500g	1000g
Art. No.	LPS0025	LPS0100	LPS0500	LPS1000

LEPURE

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