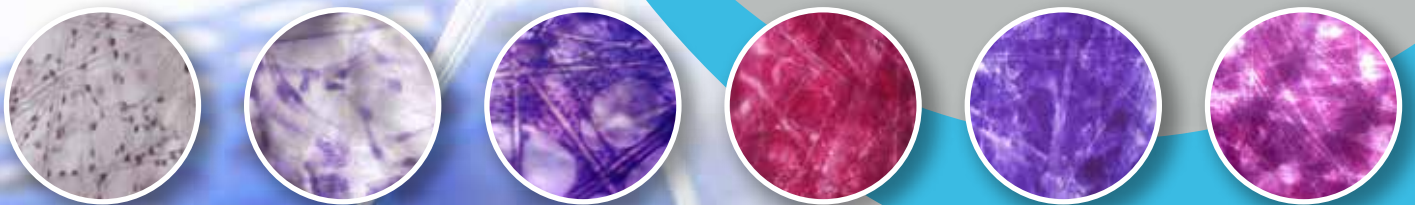




BioNOC II®

The Heart of Tide Motion
Bioreactors



BioNOC II®

The Heart of Tide Motion Bioreactors

BioNOC II® is a macroporous carrier that supports the growth of anchorage-dependent cells including animal, mammalian, and insect cells in either serum-containing or serum-free culture media. It is made of 100% pure polyethylene terephthalate (PET) nonwoven fabric manufactured according to cGMP guidelines. The special geometric design and surface treatment on the carrier enhance fluid mixing, immobilization efficiency, protection from shear forces, and nutrient transfer during cell culture.

Anchorage-dependent cells require surface in order to grow. Culture vessels such as T-flasks and roller bottles are typically used but the difficulty in scale up have led to the development of carriers. The carriers provide optimum environment for cells and are advantageous in terms of scale-up, cost reduction from serum and culture media, minimum risk of contamination, and reduction of handling process.

These macroporous carriers are used to grow, but not limited to, virus-generating or protein-producing adherent cells in a large-scale commercial production of vaccines and biologics.



TideXcell®-002

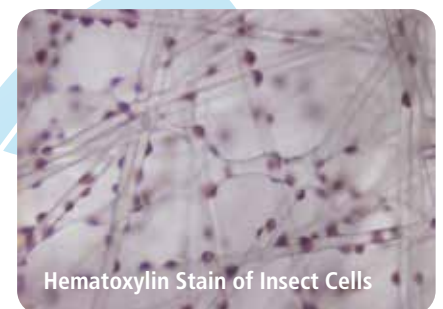
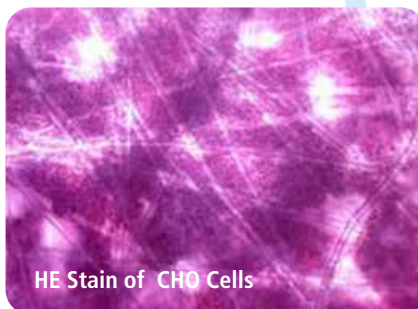
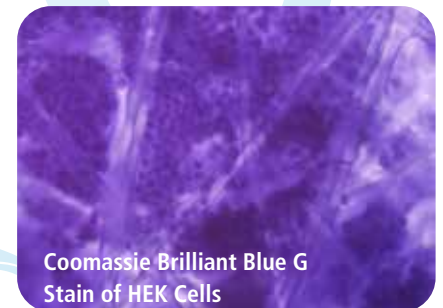
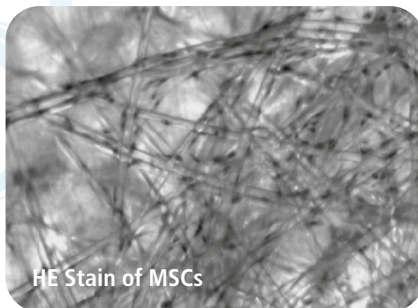
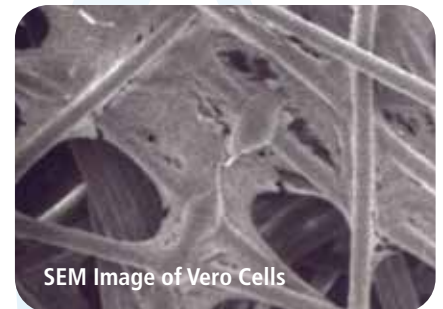
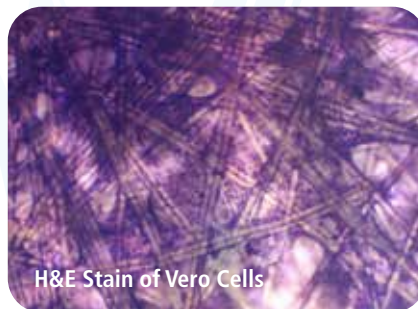
Key Features

- **Productivity:** up to $2-3 \times 10^9$ of Vero Cells in BioNOC II®
- **Surface area:** up to 2,700 cm²/g of BioNOC II®
- **Enhanced biocompatibility:** coating of attachment factors on carriers possible
- **Low particulate:** convenient for adherent cell growth for protein or viral vaccine production
- **Quality documentation:** process control and quality assurance (cGMP guideline), full support of documentation

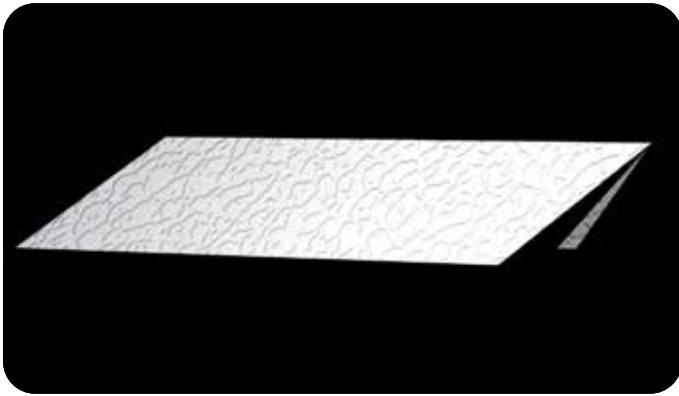
Note: Productivity and growth area vary on the cell line used.

Cultured Adherent Cell Lines

- Vero
- MDCK
- SF-9
- SF-21
- rCHO
- HEK293
- C2C12
- Hela
- MDBK
- MRC 5
- WI 38
- Marc 145
- Chick embryonic fibroblast (CEF)
- Hybridoma
- Human bone marrow-derived mesenchymal stem cells (hMSCs)



The Structure

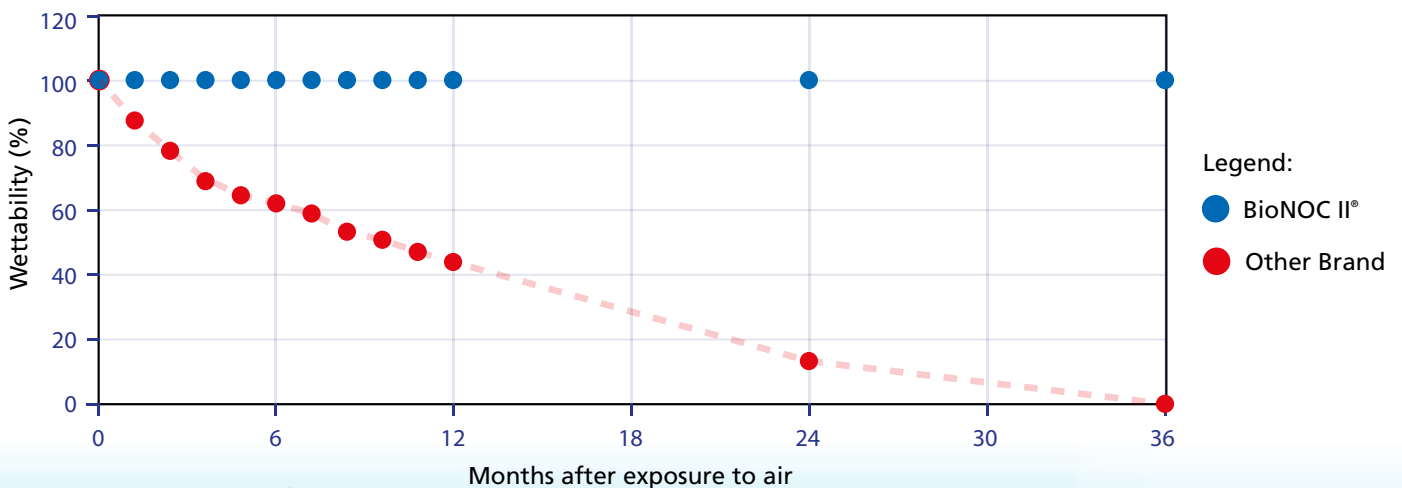


High Porosity and S/V Ratio

- High surface area to volume ratio: 160/cm
- Pore size mainly distributed between 50-200 μm
- Can yield up to $2-3 \times 10^9$ Vero cells in 5.5 grams of carriers
- Carrier thickness ~ 0.4 mm
- Allows easy exposure to culture media and aeration

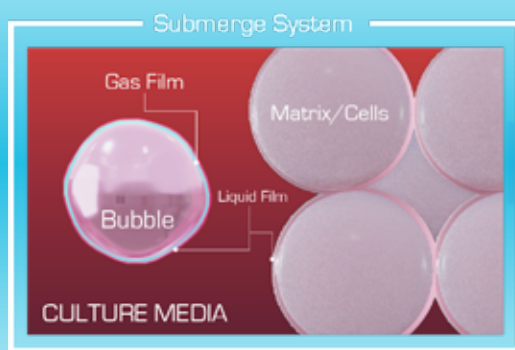
Rigid enough to support fixed bed

- High load-bearing capability to support packed-bed structure
- Increased void fraction and reduced diffusion limitation
- 45° angle generates microscopic eddy for mixing.

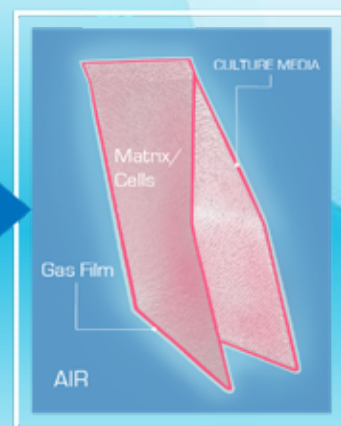


BioNOC II[®] Carriers exhibit consistent wettability for over 36 months

Esco VacciXcell Switch



Microcarrier



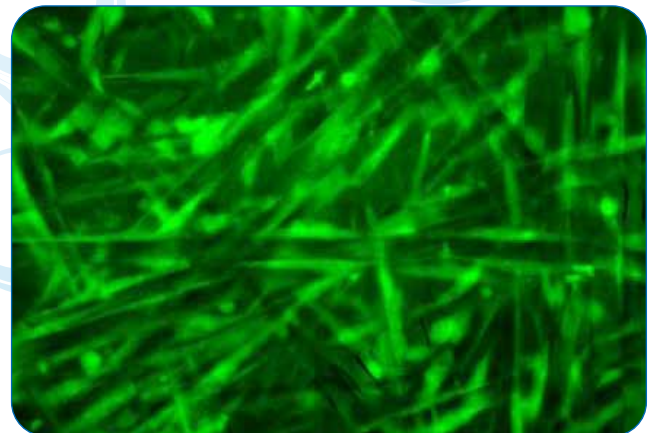
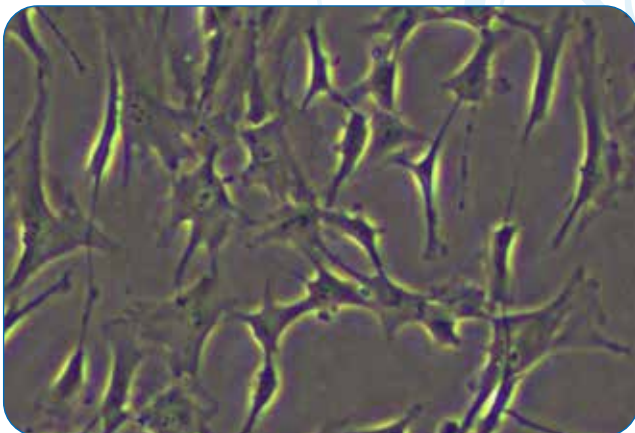
Macroporous Carrier

Cell Morphology

For adherent cell production, while cells in two-dimensional cultures grow in a monolayer culture flask or petri dish, three-dimensional cultures imitate the architecture of the parental tissue more accurately compared to 2D culture systems. These systems also have the capability to scale up rather than scale out.













Existing 2D Culture Systems	BioNOC II® 3D Culture
Single layer cell shape	Multiple layer cell shape
Morphology is sheet-like flat and stretched in monolayer	Morphology from aggregate or spheroid structures
Cell-to-cell contact is limited	Physiologic cell-to-cell contact dominates
Cells contact extracellular matrix only on the surface	Cells interact with extracellular matrix (ECM)
Culture unable to establish a microenvironment	Culture mimics an <i>in vivo</i> -like environment
Displays differential gene/protein expression as compared with <i>in vivo</i> models	Gene and protein expression levels <i>in vivo</i> present
Limited surface area available for growth at a given volume	Larger surface area capable of culturing up to 10 ⁹ cells per gram
Only offers incremental increase in the SAV	Dramatic increase in SAV

MSCs' Morphology Altered When Cultured in 2D T-flask VS 3D BioNOC II® Macrocarriers



Cell Yield and Surface Area Comparison

The surface area available for adherent cell growth in the BioNOC II® macrocarriers will depend on the type of cell line used. Some cells occupy larger space like stem cells as they stretch out as they grow, and other cell lines are relatively smaller.

								
	Model (Roller bottle equivalency)	Vol. of BioNOC II® (w/o cartridges)	Matrix volume (w/o cartridges)	T-flask (150 cm²)	Petri Dish (180 cm²)	Roller Bottles (850 cm²)	Plates (6320 cm²)	Spinner Flask *dependent on microcarrier volume
	CelCradle®	5.5 g	0.1 liters (100 mL)	100 pcs.	83 pcs.	18 – 20 pcs.	2 pcs.	10 pcs.
	TideXcell®-002	110 g	2 liters (2000 mL)	2,000 pcs.	1,667 pcs.	353 pcs.	47 pcs.	45 pcs.
	TideXcell®-010	550 g	10 liters (10,000 mL)	10,000 pcs.	8,333 pcs.	1,765 pcs.	237 pcs.	227 pcs.
	TideXcell®-020	1100 g	20 liters (20,000 mL)	20,000 pcs.	83,333 pcs.	3,529 pcs.	475 pcs.	454 pcs.
	TideXcell®-100	5500 g	100 liters (100,000 mL)	100,000 pcs.	16,667 pcs.	17,647 pcs.	2,373 pcs.	2,273 pcs.
	TideXcell®-300	16,500 g	300 liters (100,000 mL)	300,000 pcs.	250,000 pcs.	52,412 pcs.	7120 pcs.	6,818 pcs.
	TideXcell® 3,000-5,000	165,000 g - 275,000 g	3,000-5,000 liters	3,000,000 - 5,000,000 pcs.	2,500,000 - 4,166,667 pcs.	529,412 - 882,353 pcs.	71,203 - 118,671 pcs.	68,182 - 113,636 pcs.

*Specifications subject to change



Model (Roller bottle equivalency)	Vol. of BioNOC II® (w/o cartridges)	Matrix volume (w/o cartridges)	Matrix Volume (with cartridges)	Cell Seeding (Recommended -VERO)	Expected Resulting Cell Density *Vero cells in NSFM (lower density in SFM)	Cell Seeding (Recommended -MSC)	Expected Resulting Cell Density *MSCs / HEK or primary cells (eg. Hepatocytes) in SFM
BelloCell™	5.5 g	0.1 liters (100 mL)	N/A	1 x 10 ⁸ cells	3 x 10 ⁹ cells	1 x 10 ⁷ cells	2-4 x 10 ⁸ cells
TideXcell®-002	110 g	2 liters (2000 mL)	110 g/ 4x cassettes	2 x 10 ⁹ cells	2 x 10 ¹⁰ cells	2 x 10 ⁸ cells	4-8 x 10 ⁹ cells
TideXcell®-010	550 g	10 liters (10,000 mL)	412.5 g/ 12x cassettes	1 x 10 ¹⁰ cells	0.75 x 10 ¹¹ 1 x 10 ¹¹ cells	1 x 10 ⁹ cells	1.5-3.0 x 10 ¹⁰ cells
TideXcell®-020	1100 g	20 liters (20,000 mL)	825 g/ 24x cassettes	2 x 10 ¹⁰ cells	1.5 x 10 ¹¹ -2 x 10 ¹¹ cells	2 x 10 ¹⁰ cells	3-6 x 10 ¹⁰ cells
TideXcell®-100	5500 g	100 liters (100,000 mL)	3934 g/ 96x cassettes	1 x 10 ¹¹ cells	0.75 x 10 ¹² -1 x 10 ¹² cells	1 x 10 ¹¹ cells	1.4-2.8 x 10 ¹¹ cells
TideXcell®-300	16,500 g	300 liters (300,000 mL)	11.84 kg/ 296x cassettes	1 x 10 ¹² cells	2.25 x 10 ¹² 3 x 10 ¹² cells	1 x 10 ¹² cells	2-4 x 10 ¹² cells
TideXcell®	165,000 g- 275,000 g	3,000-5,000 liters	118.4 kg/ 2960-197.3 kg 4933x cassettes	3 x 10 ¹² cells	3 to 5 x 10 ¹³ cells	2 x 10 ¹² cells	4-8 x 10 ¹² cells

*Specifications subject to change

Cell Culture with BioNOC II®

Seeding Cells in BioNOC II®

1. Aseptically transfer 30 pieces of autoclaved carriers into a 50 mL tube. The number of carriers for seeding cells depends on the size of the flask to be used during cultivation.
2. Inoculate carriers by pipetting required number of cells into 50 mL tube. Please refer to Table 1 for optimal cell seeding number per carrier.

Cell type	Seeding density (Cells/carrier)
Chick embryo fibroblasts (CEF)	300,000 - 500,000
A-549	200,000 - 300,000
Chinese hamster ovary cells (CHO)	100,000 - 300,000
HEK293T / PK-15 / IBRS-2	100,000 - 300,000
Vero	100,000 - 300,000
Hybridoma (OKT3)	100,000 - 300,000
MDCK	60,000 - 120,000
Leghorn male hepatoma (LMH)	50,000 - 200,000
MARC-145	50,000
Human mesenchymal stem cells (hMSCs)	20,000 - 60,000
Human diploid cells (WI-38 / MRC-5)	15,000 - 20,000

Table 1. Optimal cell seeding density of various cell types

Note: A wide variety of cell types can be used to seed onto carriers. Check with Esco personnel for cell types not falling in this category.

3. Add media into 50 mL tube until carriers are completely submerged. pH value should be maintained between 7.0 – 7.4 (optimum pH value is 7.2).
4. Gently tilt and rotate the tube 2-3 times to allow uniform mixing of cells with carriers.
5. Incubate the 50 mL tube in upright position for 3 to 5 hours in an incubator (37°C, 5% CO₂) with cap loosened for CO₂ equilibration.
6. At 15 min intervals during the first hour, tighten the cap of the tube, gently tilt and rotate the tube (as described on step 3) to resuspend cells that may have settled. Loosen the cap and place the tube back in the CO₂ incubator.
7. During the next 2-3 hours, repeat the process (as described in step 6) at 30-minute intervals.
8. After 3 hours of incubation, transfer the 50 mL tube to the biosafety cabinet, gently tilt and rotate the tube's contents and sample 50 µl medium for cell counting. Count suspended cells remaining in the culture medium and determine % of attachment.
9. If more than 90% of attachment rate is achieved (i.e. less than 10% cells remain in the culture medium), proceed to the cell cultivation phase.

Note: The mentioned steps are for demonstration only. Please contact our local team to further support you with your cell culture process.

Cell Cultivation with BioNOC II®



Watch product video here:
Proof of Concept Tide Motion

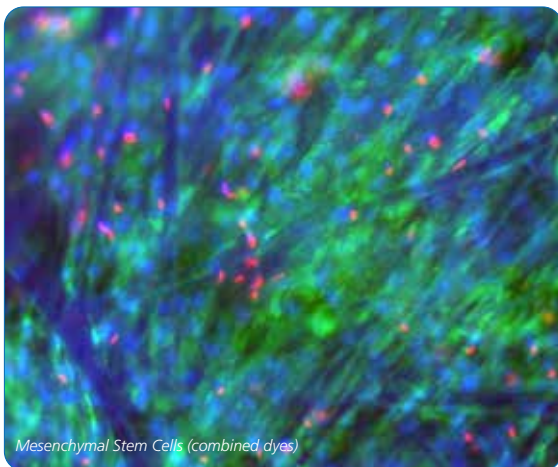


1. Position rocker in CO₂ incubator at 37°C and 5% CO₂. Adjust rocking speed to 4 cycles per min (each cycle consists of a rocking cycle of rocker position from left → right → left).
2. At the end of the attachment period, use a pair of sterile forceps to gently transfer the carriers from the centrifuge tube to a T-75 flask containing 18 mL (0.6 mL of media/carrier) of fresh medium.
3. Centrifuge the media used for inoculation to count the total unattached cells and determine the final attachment efficiency.
4. Place the flask on the rocker prepared in a CO₂ incubator maintained at 37°C and 5% CO₂ (or at preferred set-points for the cell type being cultured).
5. Conduct media change every 2-3 days or according to protocols established in your lab for the particular cell line.

Note:

1. After inoculation, the cells are mildly attached to the carriers. Be gentle while handling the carriers to prevent cells from dislodging.
2. The mentioned steps are for demonstration only. Please contact our local team to further support you with your cell culture process.

Cells Grown on and Within BioNOC II® Fiber Network



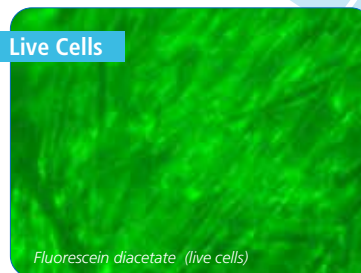
Nucleus



Phase



Live Cells



Dead Cells



Cell Harvesting with BioNOC II®



Transfer: Aseptically transfer carriers from the flask into a centrifuge tube.



After the incubation period, transfer the enzyme into a collection tube.



Repeat: Repeat steps 5 and 6 at least 3 more times with 1 mL of PBS or culture media.



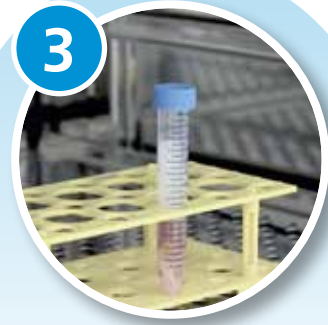
Rinse: Gently rinse the carriers with 1 mL calcium and magnesium free phosphate buffered saline (PBS) thrice (gently invert the tube 5 times for each wash).



Inhibitor addition and mechanical agitation: Add neutralization solution or PBS (depending on the enzyme used) into the centrifuge tube containing the carriers and flick the tube using the back of a forceps for 40-60 times.



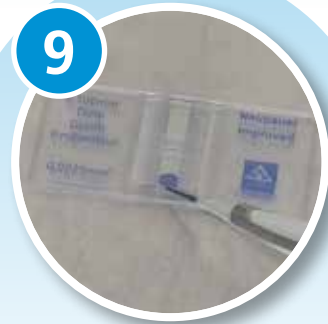
Count: Count cells or centrifuge, aspirate supernatant and resuspend cells in lower volume to obtain cell count.



Incubate: Perform enzyme dissociation. The type of enzyme to be used depends on the cell type being cultured. Incubate the carriers with the enzyme within the recommended time indicated in the protocol.



Harvest: Transfer the solution to a collection tube.



Stain: Seed cells into well-plates or flasks to check morphology and viability after harvest.

Note:

1. Different types of dyes may be used e.g. Trypan blue, Crystal Violet Dye, Fluoresce dye and more for staining to obtain better visualization of cells left on carriers post harvesting. Refrain from using colored dyes for post-harvest stains.
2. The mentioned steps are for demonstration only. Please contact our local team to further support you with your cell culture process

Specification

Function	For Culture of Adherent Cells
Material	100% non-woven polyethylene terephthalate (PET) filament
Dimension	6 mm × 10 mm strip
Surface Area / g	Up to 2,400 cm ² /g
Capacity	2-3 × 10 ⁹ (Vero Cells) of BioNOC II [®]
Default Packed Volume	15 mL/g (5.5 g for BelloCell™, 110 g for TideXcell [®] 2 L matrix vessel, 550 g for TideXcell [®] 10 L matrix vessel, 1,100 g for TideXcell [®] 20 L matrix vessel, 5,500 g for TideXcell [®] 100 L matrix vessel)
Pore Size	50 ~200 μm
Porosity	90~94%
Endotoxin Test	< 0.25 EU/mL
Bioburden Test	< 1 CFU / g
Cytotoxicity Tested	Non-toxic
Cell Line	CHO, CHO-K1, rCHO-hlgO, rC-127-TPA, HEK-293, VERO, Sf9, Hi-5, BHK-21, rBHK-Factor VIII, HepG2, Hela, Huh 7, RK-13, ST, MDCK, MDBK, 3T3, MRC-5, CEF, Human foreskin fibroblast, human muscle skeleton cell, human mesenchymal cell, human embryonic stem cell, etc
Regulation Guideline	USP Class VI, USP <87>,<83>, ISO 10993-5
Storage	Room temperature, dark area
Shelf Life	2 years
Sterilization	Tolerate autoclave (121°C), gamma irradiation (25~45 kGy), and EO sterilization

Ordering Information

Product Name	Item Code	Package
BioNOC II [®] Cell Culture Carriers (50 g)	1400018	50 grams per bottle
BioNOC II [®] Cell Culture Carriers (250 g)	1400019	250 grams per bottle
BioNOC II [®] Cell Culture Carriers (1000 g)	1400020	1000 grams per bottle

ESCO[®]
Healthcare



"Discovery to Delivery"

ESCO
VACCIX**CELL**
ADHERENT BIOPROCESSING SPECIALIST

For queries and comments, please contact Esco Vaccixcell
Technical Support team.

21 Changi South Street 1 • Singapore 486777
Tel +65 6251 9361 • mail@escovaccixcell.com
www.escovaccixcell.com

CDMO Services:

**Esco Aster Fermentation
(Plasmids)**
21 Changi South Street 1
Singapore 486777
Tel +65 6542 0833

**Esco AsterTide PD-Phase 2 CTM
(Cells/Viruses/EVs)**
#02-04 Blk 67 Ayer Rajah Crescent
Singapore 139950
Tel +65 6251 9361

**Esco AsterMavors Cellular Agriculture
and Alternative Proteins (Food)**
#03-20 Blk 71 Ayer Rajah Crescent
Singapore 139951
Tel +65 6251 9361

