ESCO VACCIXCELL
ADHERENT BIOPROCESSING SPECIALIST

Designed in Singapore

BioNOC™ II

The Heart of Tide Motion Bioreactors

ESCO® Healthcare | "Discovery to Delivery"

BioNOC™ II

The Heart of Tide Motion Bioreactors

Anchorage-dependent cells require surface to adhere 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 anchorage-dependent cells. The advantages of carriers are ease 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.

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.

TideXcell®-002

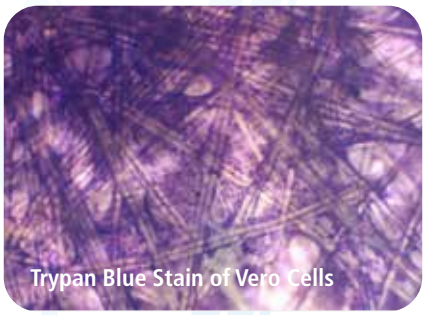
Key **Features**

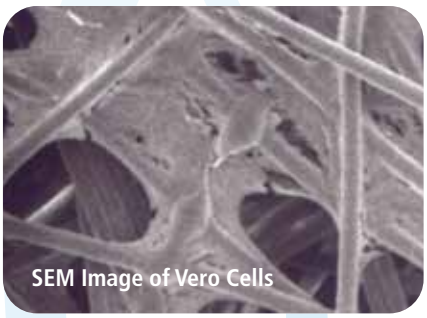
- Productivity: up to 5X10^9 cells/g of BioNOC™ II
- Growth area: up to 2,400 cm^2/g of BioNOC™ II
- Enhanced biocompatibility; coating of attachment factors on carriers possible
- Low particulates, convenient for adherent cell growth for protein or viral vaccine production
- Process control and quality assurance (cGMP guideline), full support of documentation

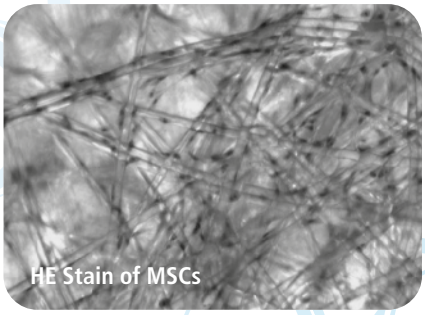
Note: Productivity and growth area vary on the cell line to be used.

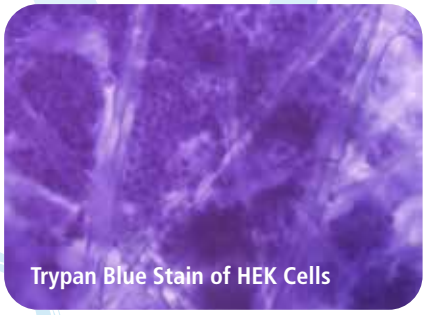
Cultured Adherent **Cell Lines**

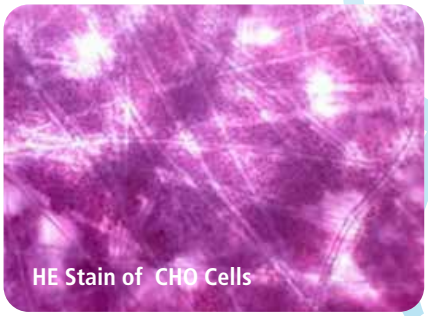
- VERO
- Mesenchymal Stem Cells (MSCs)
- Sf9
- MDCK
- MDBK
- Hela
- HEK-293
- CHO
- Hybridoma


Trypan Blue Stain of Vero Cells


SEM Image of Vero Cells

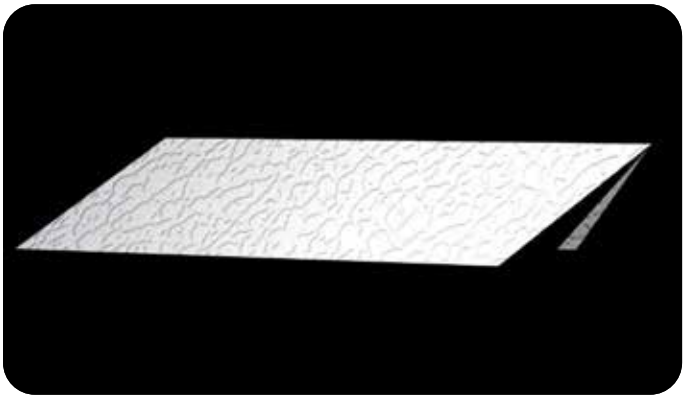

HE Stain of MSCs


Trypan Blue Stain of HEK Cells


HE Stain of CHO Cells


Hematoxylin Stain of Insect Cells

The **Structure**

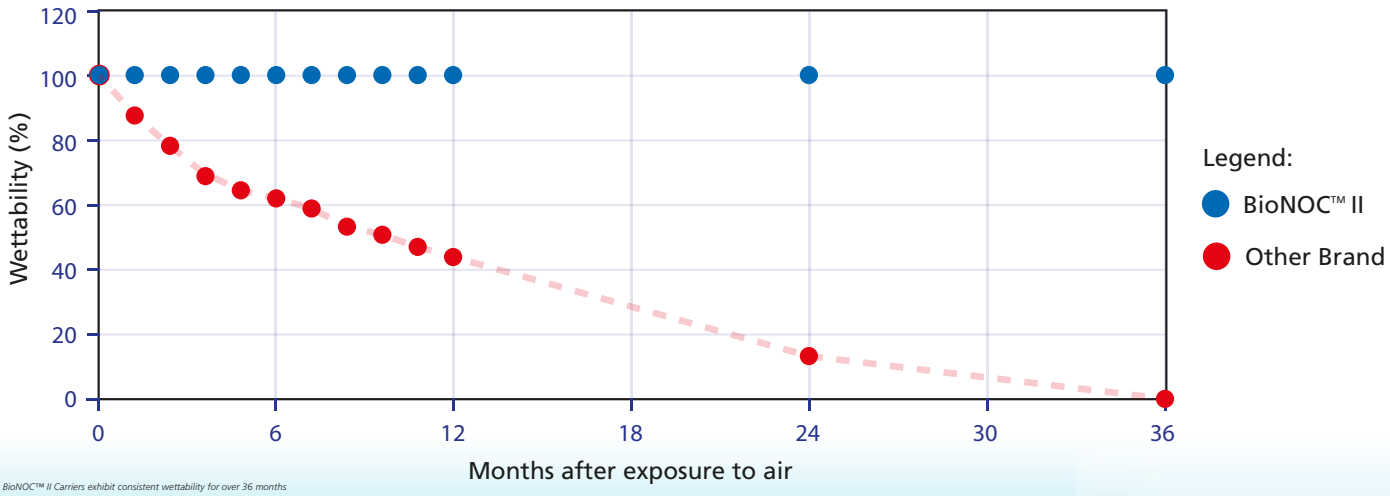


High Porosity and Thickness

- High surface area to volume ratio: 160/cm
- Pore size mainly distributed between 50-200 μm
- Can yield up to 2-3 x 10^8 VERO cells in 5.5 grams of carriers
- Fiber thickness ~0.4mm
- Allow easy exposure to culture media and aeration
- No oxygen limitation

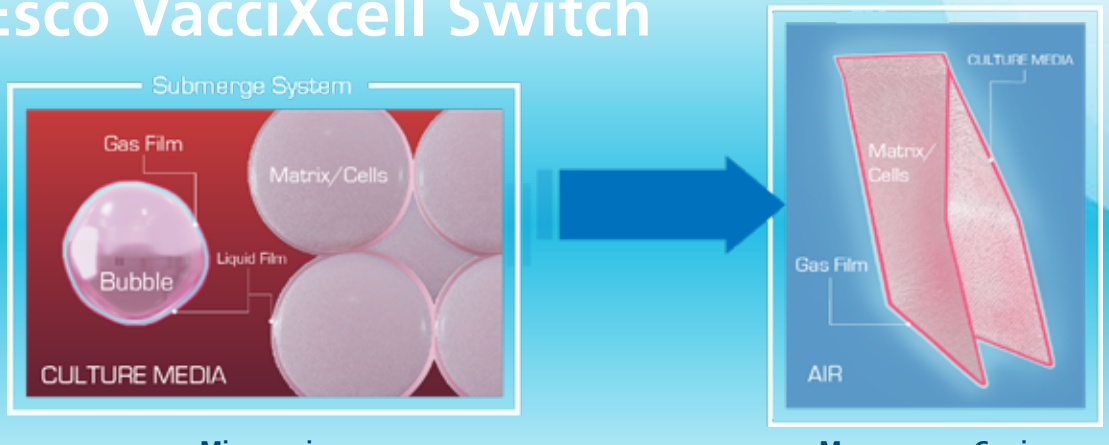
Rigid enough to support fixed bed

- 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



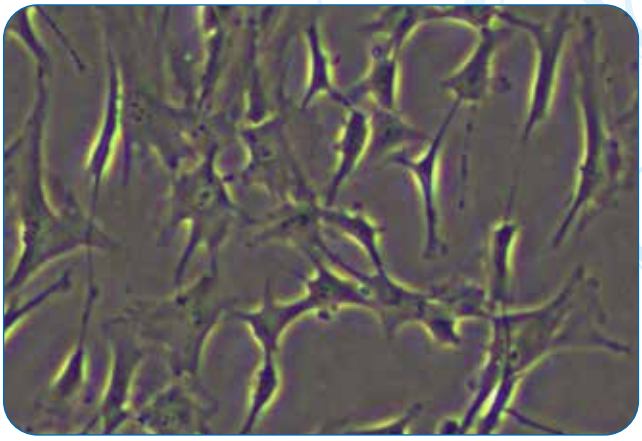
Microcarrier **Macroporous Carrier**

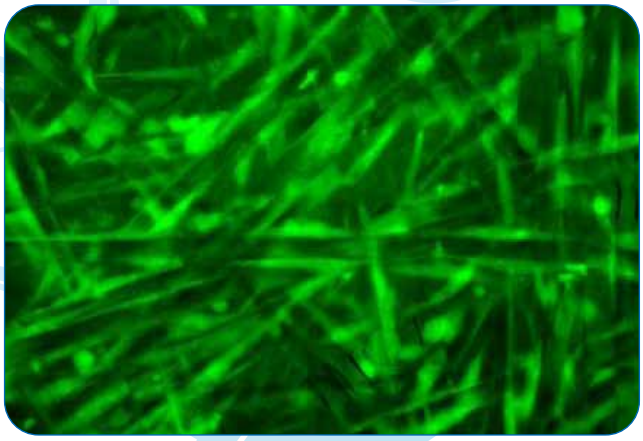
Cell **Morphology**

For adherent cells production, while cells grown in two-dimensional adherent cultures grow in a monolayer culture flask or petri dish and attaches to the surface of the system, 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 *in vivo*-like environment
Displays differential gene/protein expression as compared with *in vivo* models	Gene and protein expression levels *in vivo* 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 SA/V	Dramatic increase in SA/V

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
CelCradle™	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 100,000 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

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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 variuos 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% CO_2) with cap loosened for CO_2 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 re-suspend cells that may have settled. Loosen the cap and place the tube back in the CO_2 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

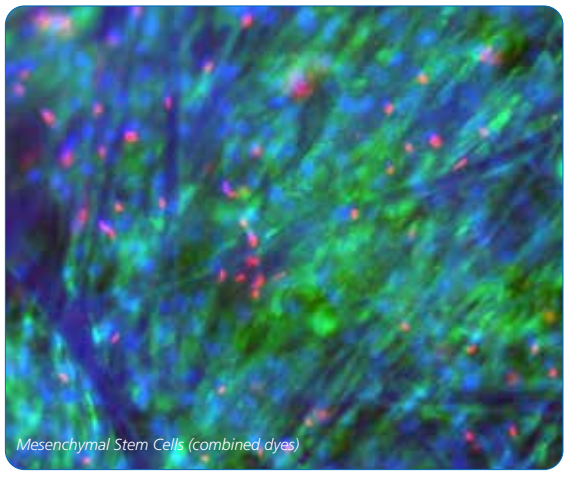
1. Position rocker in CO_2 incubator at 37°C and 5% CO_2. Adjust rocking speed to 4 cycles per min (each cycle consists of a rocking cycle of rocker position from left → right → left).
2. At the end of the attachment period, use a pair of sterile forceps to gently transfer the carriers from the centrifuge 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 CO_2 incubator maintained at 37°C and 5% CO_2 (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.

Watch product video here:
Proof of Concept Tide Motion

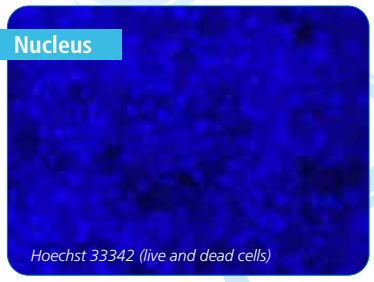
Note:

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

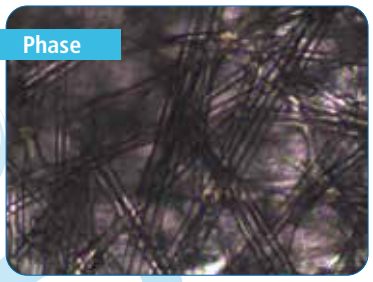


Mesenchymal Stem Cells (combined dyes)

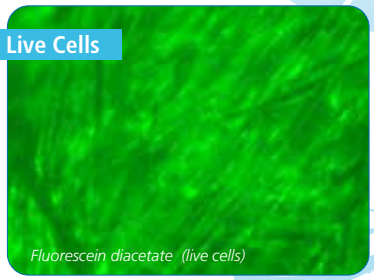


Nucleus

Hoechst 33342 (live and dead cells)



Phase



Live Cells

Fluorescein diacetate (live cells)

Dead Cells

Propidium iodide (dead cells)

Cell **Harvesting** with **BioNOC™ II**

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

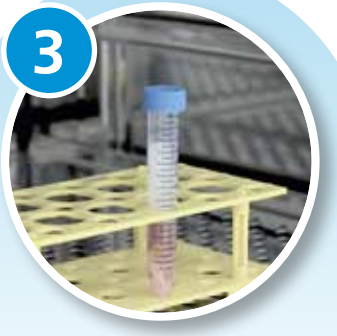
After the incubation period, transfer the enzyme into a collection tube.

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 PET filament
Dimension	5 mm × 10 mm strip
Surface Area / g	Up to 2,400 cm^2/g
Capacity	up to 5X10^8 cell/g of BioNOC™ II
Default Packed Volume	15 mL/g (5.5 g for CelCradle™, 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	Yes, pyrogen-free
Cell Line	CHO, CHO-K1, rCHO-hIgO, 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 kGy), and EO sterilization

Ordering Information

Product Name	Item Code	Package
BioNOC™ II Cell Culture Carriers (50 g)	1400018	50 gram per bottle
BioNOC™ II Cell Culture Carriers (250 g)	1400019	250 gram per bottle
BioNOC™ II Cell Culture Carriers (1000 g)	1400020	1000 gram per bottle

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

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