



# EndoGo™ XF

A defined, xeno free (XF) culture medium specially designed to support long-term expansion of large and small vessels endothelial cells from various sources.

## Instructions for Use

### Medium Components

Product Description	Storage	Cat. No.	Size
EndoGo™ XF Basal Medium	-10 to -20°C	05-400-1A	500 ml
EndoGo™ XF Supplement Mix	-10 to -20°C	05-410-1-25	2.5 ml

#### Notes:

1. The medium required the use of human AB serum (off the clot) or human platelet lysate
2. Some cells will require pre-coating of culture vessel (e.g. with human Fibronectin Solution)

### Introduction

Endothelial cells (EC) form the inner lining of blood vessels, termed Endothelium. They provide a selective permeability barrier and an anticoagulant barrier between the vessel wall and blood.

Their unique location at the interface between the blood and surrounding tissue allows EC to detect local changes and blood-borne signals. EC react by producing a wide range of vasoactive substances that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation. Additionally, EC are pivotal in cancer research, angiogenesis and vasculogenesis. Consequently, EC have become a key element in research, tissue engineering and cell therapy.

### Product Description

EndoGo™ XF is a novel XF culture medium specially designed for long-term expansion of large and small vessels EC from various sources.

Before use, supplementation with 2-5% of human AB serum (off the clot) or human platelet lysate (hPL) is required.

EndoGo™ XF does not contain any non-human origin ingredients e.g., Bovine Brain Extract (BBE).

The medium provides optimally balanced nutritional environment that selectively promotes proliferation of normal human EC, while maintaining typical cobblestone-like cell morphology, phenotypic surface marker profile, and angiogenic differentiation potential.

EndoGo™ XF supports microvascular EC (MVEC) from blood and lymph vessel as well as EC derived from: dermal, cardiac, lung, bladder and adipose tissues.

In addition, EndoGo™ XF supports EC from arterial or venous vessels (e.g. HUVEC).

No adaptation is required for the transition from bovine serum-containing medium to EndoGo™ XF. It is recommended for use with Human Fibronectin Solution (cat. no. 05-750- 1) for optimal attachment, spreading and proliferation of cells.

For optimal cell passage and long term culture of cells, it is recommended to use Recombinant Trypsin Solution with EDTA (cat. no. 03-079- 1) and inhibition with Soybean Trypsin Inhibitor (SBTI), (cat. no.03-048-1).

## Medium Features

- Xeno-free medium: all components are from non-xenogenic origin, including proteins.
- Enables culture of human macro and micro-vascular EC from various sources.
- Should be supplemented with off the clot human AB serum or human platelet lysate.
- Contains L-Alanyl-L-Glutamine.
- Does not contain antibiotics.

## Precautions and Disclaimer

Do not use beyond the expiration date indicated on the product label.

## Storage and Stability

EndoGo™ XF Basal Medium should be stored at -10 to -20°C.

Upon thawing the medium may be stored at 2-8°C for 1 month protected from light.

EndoGo™ XF Supplement Mix should be stored at -10 to -20°C.

Shelf Life: Refer to product label for expiration date.

## Complete Ready-to-Use Medium Preparation

Frozen EndoGo™ XF Basal Medium should be thawed at room-temperature or at 2-8°C. If precipitants appeared after thawing warm for few minutes in 37°C water bath and swirl gently, do not refreeze.

Frozen EndoGo™ XF Supplement Mix should be thawed on ice or at 2-8°C. Avoid repeated freeze-thaw cycles (up to two freeze – thaw cycles max.).

For the preparation of the complete medium, aseptically add 2.5ml of EndoGo™ XF Supplement Mix to 500ml of EndoGo™ XF Basal Medium.

Add 2-5% of human AB serum (off the clot) (e.g. Access Biologicals) or pooled human platelet lysate (e.g. Cook Regentec, Stemulate™ G34934).

Complete medium may be stored at 2-8°C protected from light for up to 30 days.

Complete EndoGo™ XF must be warmed to room temperature (15-30°C) before use. To ensure stability of the medium, warm only the amount needed.

## Preparation of Pre-Coated Culture Dishes with Fibronectin Solution (cat. no. 05-750-1)

The optimal Human Fibronectin coating concentration is cell dependent and may vary between 0.5-5 µg/cm<sup>2</sup>. We recommend starting a coating concentration of 2µg/cm<sup>2</sup>.

1. Determine the amount of Fibronectin needed to coat culture vessels by multiplying the total surface area (cm<sup>2</sup>) by the desired coating concentration (µg/cm<sup>2</sup>). Calculate the required coating volume of Human Fibronectin Solution.

### For example:

To coat 10 cm<sup>2</sup> at 2µg/cm<sup>2</sup> - 20µg of Fibronectin is needed.

The concentration of Human Fibronectin Solution is 1mg/ml, therefore, 20 µl is required.

2. Dilute Human Fibronectin Solution (cat. no. 05-720-1) in sterile DPBS w/o Ca<sup>++</sup> and Mg<sup>++</sup> (cat. no. 02-023-1) and coat the culture surface with a minimal volume. The volume should be adequate for covering the desired well or plate. Refer to table 1 for minimal recommended volumes.
3. Gently agitate the coated dish. Verify complete covering of the surface vessel.
4. Incubate for at least 30 minutes in a humidified CO<sub>2</sub> incubator (37°C).
5. Aspirate the Human Fibronectin Solution and discard.
6. Immediately, wash the culture vessel with DPBS w/o Ca<sup>++</sup> and Mg<sup>++</sup> (cat. no. 02-023-1) and immediately add complete culture medium. It is critical that the coating does not dry out. Alternatively, coated plates may be stored for later use at 2-8°C up to one week.  
After washing the vessel, add sufficient volume of DPBS, wrap with Parafilm® and lay flat at 2-8°C up to one week. Prior to use, pre-warm the culture vessel to room temperature.

### Recommended volumes for the coating procedure

Culture vessel	Surface area cm <sup>2</sup>	Volume of diluted Fibronectin Solution
96-well plate	0.3	0.05 ml
24-well plate	1.9	0.2 ml
12-well plate	3.9	0.4 ml
6-well	9.6	1 ml
T25 Flask	25	2.5 ml
T75 Flask	75	7.5 ml

### Notes:

Longer coating time with Human Fibronectin Solution in a humidified CO<sub>2</sub> incubator at 37°C, will not decrease cell attachment and spreading performance (up to 72 hours).

## Culturing of Endothelial cells in EndoGo™ XF complete medium

### A. Recovery of Cryopreserved Endothelial cells

1. Pre-warm 5-10 ml of complete EndoGo™ XF at 37°C in a 50 ml conical tube.
2. Rapidly thaw frozen vial of EC in a 37°C water bath, with gentle agitation until a small amount of ice remains.
3. Slowly add (drop by drop while gently swirling the tube) the cells into the pre-warmed complete EndoGo™ XF.
4. Centrifuge cells at 200-300 g for 5 minutes at room temperature.
5. Remove supernatant and re-suspend cell pellet in 0.5-1 ml of complete EndoGo™ XF.

6. Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay).
7. Add the desired volume of complete EndoGo™ XF.
8. Transfer the cells into pre-coated culture vessel (see above). Seeding densities should be 5,000 cells/cm<sup>2</sup>. Optimal seeding densities vary from one cell type to another and should be determined. 4,000-10,000 cells/cm<sup>2</sup> should work well for most EC.
9. Incubate in a humidified CO<sub>2</sub> incubator at 37°C.
10. Perform medium change every 2-3 days until culture reaches 80% coverage and ready for passage.
5. Incubate for 2-4 minutes at room temperature and verify cell detachment using microscope (Incubation at 37°C will accelerate detachment).
6. Following detachment, add 5-10 ml of pre-warmed EndoGo™ XF or 1xSBTI solution.
7. Collect cell suspension into a sterile tube and re-wash the culture dish as necessary to collect remaining cells.
8. Centrifuge cells for 5 minutes at 200-300g at room temperature. Carefully discard the supernatant.
9. Re-suspend cells with complete EndoGo™ XF.
10. Re-seed cells into pre-coated culture dish at 4000-7000 cells/cm<sup>2</sup>.
11. Incubate in a humidified CO<sub>2</sub> incubator at 37°C.
12. Re-feed cells with fresh warmed complete EndoGo™ XF every 2-3 days.

**Note:**

It is possible to avoid the centrifugation step after thawing. In this case skip steps 1.4 and 1.5 and transfer the thawed cells (from Step 1.3) directly into the pre-coated culture vessel with the required volume of complete medium, at a ratio of at least 1:10 (for the dilution of the DMSO). Perform a complete medium change after 24 hours.

**Sub-Culturing Human Endothelial Cells**

EndoGo™ XF was developed for optimal proliferation of EC from a variety of sources (umbilical cord, blood, dermal, lung, bladder and adipose). The variety of sources and the variability of donors may influence EC proliferation rate. For optimal proliferation of EC in EndoGo™ XF, it is recommended to seed cells at a concentration of 4000-7000 cell/cm<sup>2</sup>, re-feed cells with fresh warmed complete EndoGo™ XF every 2-3 days and subculture when the cells reach up to 80% confluence.

**Note:** The following sub-culture protocol is based on using Recombinant Trypsin with EDTA (cat. no. 03-079-1), and inhibition with Soybean Trypsin Inhibitor (SBTI), (cat. no.03-048-1).

**B. Sub-Culturing Protocol**

1. Pre-equilibrate EndoGo™ XF medium at humidified CO<sub>2</sub> incubator at 37°C for 30 minutes prior to cell seeding.
2. If using SBTI solution: dilute the SBTI Solution (cat. no. 03-048-1) 1:50 using sterile DPBS and gently mix using a pipette. The diluted SBTI solution may be stored at 2-8°C for up to 60 days.
3. Remove culture medium and gently wash once with DPBS w/o Ca<sup>++</sup>, Mg<sup>++</sup> (cat. no. 02-023-1).
4. For T25 culture flask add 1ml of Recombinant Trypsin with EDTA solution (cat. no. 03-079-1). For any other culture dish, the appropriate volume should be adjusted.

**Note:**

High confluence of cells or higher passage will result in slower detachment and will require higher volume of Recombinant Trypsin with EDTA solution.

**C. Cryopreservation of Endothelial Cells**

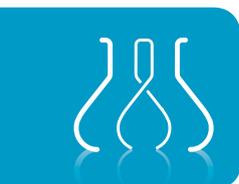
Rapidly re-suspend cell pellet with ice cold Serum-Free Freezing Medium (cat. no. 05-065-1) (recommended concentration between 0.25-1x10<sup>6</sup> cells/ml, 1ml/vial). Immediately place the cryo-vials in appropriate freezing container (e.g., "Mr. Frosty") and place at -80°C overnight. Transfer the cryo-vials into a liquid nitrogen storage tank.

**Quality Control**

EndoGo™ XF performance is tested for optimal maintenance and expansion of HDMEC. Additional standard evolutions are pH, osmolality, endotoxins and sterility tests.

**Auxiliary products**

Product Name	Cat. No.	Size	Storage
Human Fibronectin Solution	05-750-1 F	1ml	2-8°C
	05-750-1 H	5ml	
Recombinant Trypsin EDTA Solution	03-079-1B	100ml	RT
	03-079-1C	20ml	
Soybean Trypsin Inhibition	03-048-1C	20ml	-20°C
SF Cell Freezing Medium	05-065-1B	100ml	2-8°C
	05-065-1A	500ml	
Dulbecco's PBS (w/o Ca & Mg)	02-023-1B	100ml	2-8°C
	02-023-1A	500ml	



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Biological Industries  
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Biological Industries Israel Beit Haemek Ltd.  
Kibbutz Beit Haemek 2511500, Israel  
T.+972.4.9960595 F.+972.4.9968896  
Email: info@bioind.com  
[www.bioind.com](http://www.bioind.com)

