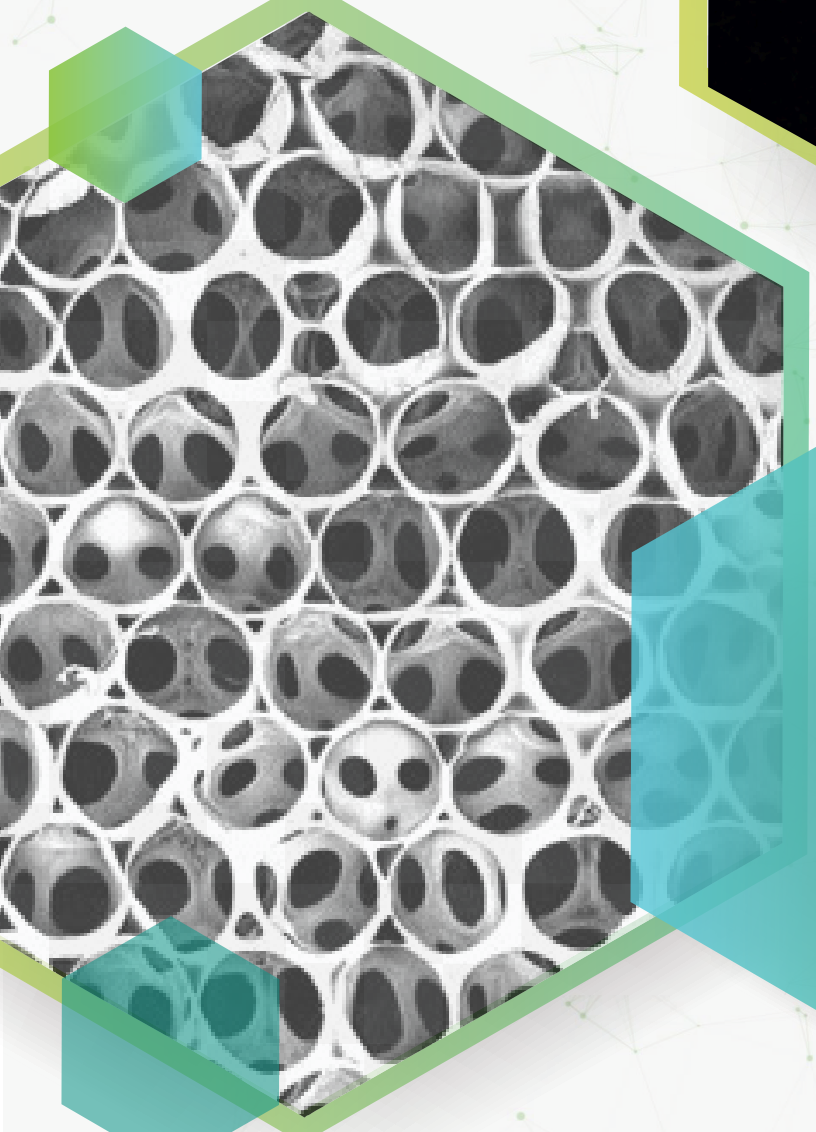

UniTantrix® Dissolvable Microcarriers

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IFU-003 Rev.02

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Instruction for use

UniTantrix® Dissolvable Microcarriers

Sterilized by gamma irradiation

Note:

- UniTantrix® Dissolvable Microcarriers is only for *in vitro* use.
- Ship and store at +10 to +35 °C.
- It shall be used up after opening the bottle.

Instruction for use

※ The following instruction for use (IFU) to demonstrate how to use 2 g/L of UniTantrix® in a 100 mL bioreactor as an example. Generally, the concentration of UniTantrix® microcarriers used (g/L), cell seeding density (no. of cells/mL of UniTantrix®) or amount of Trypsin-EDTA (or TyrpLE™) added to dissolve UniTantrix® are suggested for a 100 mL bioreactor in the IFU which can be proportionally scaled up to other volumes of your desired bioreactors.

Materials and Equipment Needed

- UniTantrix® Microcarriers (surface area: ~6,000 cm^2/g)
- 125 mL siliconized BELL-FLO™ spinner flasks or 125 mL Corning® ProCulture® glass spinner flask (Whenever the glass spinner flask is used, the inside surface of the vessel should be siliconized to prevent the microcarriers attached to glassware surface)
- Cimarec™ Biosystem Slow-Speed Stirrer for cell culture agitation
- Cell culture medium for specific cell needs
- Sterile 1 x PBS (without $Ca2^+$ and $Mg2^+$)
- 0.25% Trypsin-EDTA (or 1 x TrypLE™)
- 37°C, 5%CO_2 cell incubator

UniTantrix® Preparation

UniTantrix® Hydration and Culture Medium Equilibration.

1. Weigh 0.2 g UniTantrix® in siliconized glass spinner flask, and add to 100 mL 1xPBS.
2. Autoclave at 121°C for 20 minutes to hydrate UniTantrix®.
3. Cool down to room temperature before use.
4. Remove the PBS, add 30~50 mL fresh 1xPBS and mix. Repeat the wash step twice.
5. Replace PBS, add 50 mL fresh cell culture medium.
6. Place the spinner flask in the incubator at 37 °C and equilibrate at 40 rpm for at least 10 minutes.

Note:

a. hydrated microcarriers in PBS can be stored at 4°C for 14 days.

b. Pipetting carefully and not to aspirate microcarriers.

Instruction for use

Cell Seeding, Attachment and Expansion

A. Human Mesenchymal Stem Cells (hMSCs)

1. Trypsinize hMSCs from culture vessels and seed 6 x 10^6 (6 x 10^4/mL or 5,000 cells/cm^2) of total cell number into equilibrated spinner flasks containing UniTantrix® microcarriers.
2. Stir at 40 rpm for 40 seconds then 0 rpm for 4 hours at 37°C, 5%CO_2 cell incubator for cell attachment.
3. Add fresh cell culture medium to 100 mL of the working volume. And agitation condition was maintained at 40 rpm for cells expansion.
4. To avoid the cells-microcarrier aggregation and the stirring speed was adjusted to maintain microcarriers fully suspended during the cell's expansion (gradually increased to 50-80 rpm).

B. Vero Cells

1. Trypsinize Vero cells from culture vessels and seed 8 x 10^6 (8 x 10^4/mL or 6,600 cells/cm^2) of total cell number into equilibrated spinner flasks containing UniTantrix® microcarriers.
2. Stir at 40 rpm for 40 seconds then 0 rpm for 4 hours at 37°C, 5%CO_2 cell incubator for cell attachment.
3. Add fresh cell culture medium to 100 mL of the working volume. And agitation condition was maintained at 40 rpm for cells expansion.
4. To avoid the cells-microcarrier aggregation and the stirring speed was adjusted to maintain microcarriers fully suspended during the cell's expansion (gradually increased to 50-65 rpm).

Note:

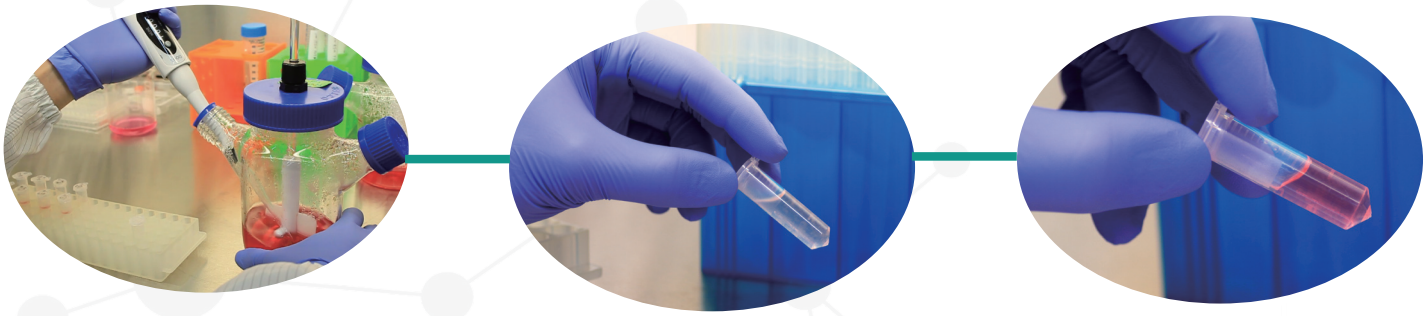
a. Stirring speed should be adjusted promptly to adapt various cell types.

b. Culture medium exchanges were performed depending on nutrient consumption and metabolite accumulation and replaced 65~75% of the working volume.

Visualization on Cell Expansion

1. Homogeneously take 1mL of culture solution and transfer to a well-plate.
2. Visualizing the cells on microcarriers under an inverted optical microscope or staining the cells with a live cell fluorescent stain to observe cell morphology.

Instruction for use



Cell Counting

1. Take 1 mL of culture solution homogeneously into a microcentrifuge tube by slowly aspirating while the spinner flask was continuously shaking.
2. Allow microcarriers to settle and gently remove cell culture medium without disturbing microcarriers.
3. Wash microcarriers with 1xPBS at least 3 times.
4. Add 1 mL 0.25% Trypsin-EDTA (or TrypLE™).
5. Place in the 37°C incubator for 10~15 minutes (20~30 minutes for TrypLE™) for UniTantrix® dissolution (Once cells are detached, the harvesting solution should be diluted with a fresh culture medium to prevent cell damage).
6. Count cell numbers to estimate cell growth.

Cell Harvesting

1. Allow total UniTantrix® microcarriers were settled down (at least 10 minutes).
2. Wash microcarriers with 1 x PBS at least 3 times.
3. Add 30 mL (20-30% of the working volume) of 0.25% Trypsin-EDTA (or TrypLE™).
4. Stir at 100~120 rpm for 15~20 minutes at 37°C (30~40 minutes for TrypLE™) for UniTantrix® dissolution (Once cells are detached, the harvesting solution should be diluted with a fresh culture medium to prevent cell damage).
5. Place the harvesting cells into a 50mL centrifuge tube.
6. Centrifuge at 1,200~1,500 rpm for 5~10 minutes.
7. Discard the supernatant and add a fresh culture medium to harvest the total cells.

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