



# Millicell® HY Flasks

## T-600 and T-1000

### User Guide

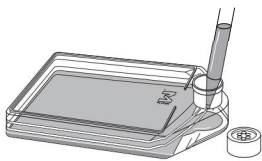
## Introduction

Millicell's Millicell HY T-600 and T-1000 multilayer flasks offer many features and benefits for the growth of large quantities of cells. These sterile, disposable flasks incorporate a multilayer design that increases the surface area for cell growth in a footprint similar to traditional T-flasks. Cell seeding density and media volume are linearly scalable based on surface area. Millicell HY Flasks have easy pipette access for aspirating and dispensing, and allow for pouring if desired.

## Protocol

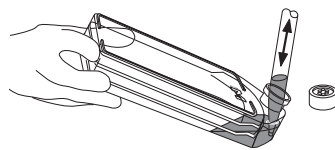
### Cell Seeding

#### FILL



1. Fill the Millicell HY T-600 or T-1000 Flask with the desired growth media.
2. Add desired number of cells to achieve typical seeding density.

#### MIX



3. Mix the cells and media by raising the end of the flask and pipetting the solution up and down several times until a homogeneous suspension is achieved.

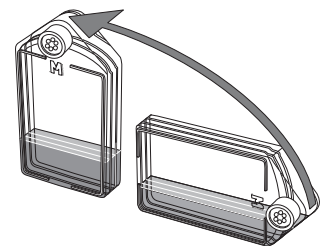
**Note:** Failure to adequately mix cells into a homogeneous suspension may result in an uneven distribution of cells across the layers of the Millicell HY Flask.

#### EQUILIBRATE



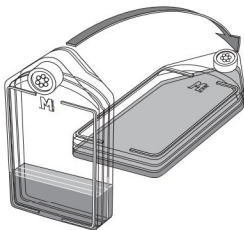
4. Briefly lay the flask on its side to equilibrate the liquid volume equally among the layers of the flask.

#### PARTITION



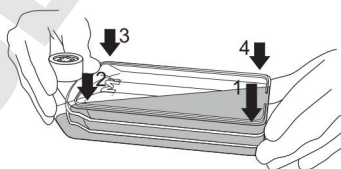
5. Tilt the flask on its end to partition the liquid volume for each layer of the flask.

#### DISTRIBUTE



6. Lay the flask down to spread the cell suspension evenly across the entire surface of each layer.

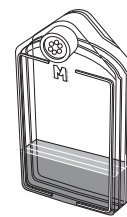
#### WETTING (OPTIONAL)



7. Depending on the volume it may be necessary to rock the flask on all four corners to ensure that the entire surface wets.

**Note:** If the liquid volume on any of the layers changes during the wetting procedure (e.g., spilling of liquid from top layer to lower layers), repeat steps 4–6 to ensure even distribution of liquid among all layers.

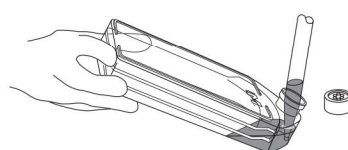
#### TRANSPORT



8. Return the flask to a vertical position when transporting to the incubator. Lay the flask flat in the incubator (as in step 6) and culture cells under appropriate conditions.

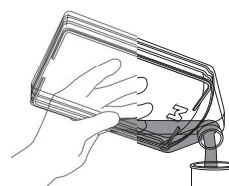
### Media Exchange

#### ASPIRATE



OR

#### POUR



9. If exchange of the media is required, either aspirate or pour the media from the Millicell HY T-600 or T-1000 Flask.

**Note:** Due to the larger surface area of the Millicell Flasks, it may be necessary to let the flask stand on its side for several minutes following each aspiration so that all residual liquid pools to a common point, allowing for a second aspiration to completely remove the remaining liquid.

10. Add appropriate volume of fresh media, and repeat steps 4–8.

## Protocol, continued

### Cell Harvest



- Remove media as in step 9.
- Add desired amount of appropriate wash solution (e.g., PBS or 0.02% EDTA) to the flask and repeat steps 4–7.
- Remove wash solution as in step 9.
- Add desired amount of dissociation enzyme (e.g., 0.25% trypsin/EDTA or equivalent) according to preferred protocol, repeat steps 4–8, and incubate. Volume of dissociation enzyme required per cm<sup>2</sup> does not need to be altered from T-flask protocol.  
**Note:** Ensure that all cells have completely dissociated from the surface of the flask via observation under microscope. Failure to allow complete dissociation of cells from culture surface (such that all cells are freely floating) may result in reduced cell yields.
- If using trypsin, add desired volume of inactivating solution, such as serum-containing media.
- Collect cells either by aspirating or pouring, as in step 9.

## Properties

Millicell HY Flasks are tissue culture treated and sterilized by gamma irradiation (all components), ready for use as received. The flasks are non-cytotoxic and non-pyrogenic; endotoxin levels are  $\leq 20.0$  endotoxin units per device when tested for bacterial endotoxins according to the USP. Millicell HY Flasks are for research use only.

## Specifications

### Materials of Construction

|          |   |
|----------|---|
| Flask    | Polystyrene                |
| Cap      | High density polyethylene  |
| Cap vent | 0.2 $\mu$ m polypropylene   |

### Dimensions

|                        | T-600                 | T-1000                |
|------------------------|-----------------------|-----------------------|
| Length                 | 22.2 cm               | 22.2 cm               |
| Width                  | 12 cm                 | 12 cm                 |
| Height (excluding cap) | 3.2 cm                | 5.1 cm                |
| Height (including cap) | 5.5 cm                | 7.4 cm                |
| Surface area           | 596.4 cm <sup>2</sup> | 984.4 cm <sup>2</sup> |

|                                 |            |            |
|---------------------------------|------------|------------|
| <b>Recommended Media Volume</b> | 120–180 mL | 200–300 mL |
|---------------------------------|------------|------------|

## Troubleshooting

| Issue   | Solution   |
|---|--|
| The media drains down to the lower levels of the flask during incubation. | Make sure that the incubator is level.<br>Do not exceed recommended media volume.  |
| The media is touching the top/lid of the flask.                           | This occurs when using $\geq 0.3$ mL/cm <sup>2</sup> of media, or media with high viscosity (e.g., 20% serum). Millipore's scientists have demonstrated that this will not impact overall cell growth/viability. To minimize this problem, reduce the media volume or serum content. |



Millicell HY Flasks have been tested for use in stem cell research applications. The tests involved growing 129/S6 mES cells in Millicell HY Flasks for 5 passages, then confirming pluripotency by cell morphology and staining for SSEA-1 and Oct-4.

Recognizing the diversity of opinions regarding embryonic stem cell research, Millipore is dedicated to conducting its business in an ethical and scientifically responsible manner. We are committed to our customers, shareholders, employees, and the global research community in our development of the highest-quality, state-of-the-art products and services for this emerging field.

## Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you, or go to our web site at [www.millipore.com/offices](http://www.millipore.com/offices) for up-to-date worldwide contact information. You can also visit the tech service page on our web site at [www.millipore.com/techservice](http://www.millipore.com/techservice).

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## Product Ordering Information

This section lists the catalogue numbers for the Millicell HY Flasks and related products. See the Technical Assistance section for information about contacting Millipore. You can also purchase Millipore products on-line at [www.millipore.com/products](http://www.millipore.com/products).

| Description  | Cat. No.  | Qty/Pk |
|--|-----------|--------|
| <b>Millicell HY Flasks</b>   |           |        |
| Millicell HY Flask T-600, sterile  | PFHYS0616 | 16     |
| Millicell HY Flask T-1000, sterile                                       | PFHYS1008 | 8      |
| <b>Related Products</b>  |           |        |
| Stericup® Sterile Filter System, 500 mL, 0.22 $\mu$ m with PES membrane  | SCGPU05RE | 12     |
| DMEM 1X, Liquid, w/ 1,000 mg/L Glucose, L-Glutamine, and Sodium Pyruvate | SLM-019-B | 500 mL |
| 6-well Cell Culture Plate, tissue culture treated, sterile               | PIMWS0650 | 50     |
| 12-well Cell Culture Plate, tissue culture treated, sterile              | PIMWS1250 | 50     |
| 24-well Cell Culture Plate, tissue culture treated, sterile              | PIMWS2450 | 50     |
| Millex® 33 mm Sterile Filter Unit, 0.22 $\mu$ m PES membrane             | SLGP033RS | 50     |
| Ecostand™ Tube Holder, 15 mL   | SC15TB001 | 1      |
| Ecostand Tube Holder, 50 mL  | SC50TB001 | 1      |

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