Harvesting of cells

The protocol for harvesting cells from a multilayer device such as CELLdisc™ which does not allow for direct platelet access varies slightly from your standard protocol. Especially as the detachment of cells can be visualized microscopically only for the bottom layer of a CELLdisc™ with one, four or maximum eight layers. For a 4-layer CELLdisc™ this results in 28-40 ml which has to be equilibrated as described above.

Remove PBS either by pouring or aspiration. Thereafter add 7-10 ml of your enzymatic dissociation agent containing media and resuspend cell pellet in fresh media. Stop the enzymatic reaction by adding the same amount of serum-containing media or an appropriate inhibitor to the CELLdisc™, equilibrate the liquids and mix the solutions by gently tilting the CELLdisc™ back and forth. Thereafter cell suspension can be harvested for further processing by pouring or aspiration.

If removal of the enzymatic agent is desired, then spin cell suspension at 100g for 5 minutes. Remove the dissociation agent containing media and resuspend cell pellet in fresh media.

The only adaption required is based on growth area and cell numbers per layer. The use of chelating agent to harvest these cells from a standard Greiner Bio-One cell culture disposable, as described in surface treatments and basic materials are identical for these vessels, standard protocols can be conver- ted. The only adaption required is based on growth area and cell numbers per layer. The use of chelating agents such as EDTA in addition to the enzyme (hypos, papain etc.) may improve cellular detachment. Remove cultivation media either by pouring or aspiration as described above. Wash cells once with PBS or an equivalent buffer using about 20 ml per layer. Follow the same protocol as for CELLdisc™ filling to distribute the buffer through all layers. Then tilt the CELLdisc™ slowly back and forth to gently rinse each cell layer and remove all traces of media.

Remove PBS either by pouring or aspiration. Thereafter and 7-10 ml of your enzymatic dissociation agent per layer. For a 4-layer CELLdisc™ this results in 28-40 ml which has to be equilibrated as described above.

Inoculate CELLdisc™ at 37 °C and 5 % CO2 for 3-5 minutes. Tapping CELLdisc™ from the side can accelerate cellular detachment. Strong adherent cells might need longer incubation or stronger tapping. For easy and secured CELLdisc™ stacking the CELLevator™ allows storage of the single layer CELLdisc™ back and forth. Thereafter cell suspension can be harvested for further processing by pouring or aspiration.

For easy and secured CELLdisc™ stacking the CELLevator™ is positioned within the circum- ferential rim of the top plate of the CELLdisc™. Another CELLdisc™ then be positioned on top of the CELLdisc™.

The single layer CELLdisc™ (CD1), in contrast to the larger multi layered versions, allows easy monitoring of cell growth, cell morphology and confluency. As conditions within the single layer CELLdisc™ will be identical to those within larger units, the CD1 can be used to anticipate when media changes are required and harvesting is recommended in any larger units being cultivated alongside the CD1. To ensure that the ambient conditions for the reference CD1 and this multi layer CELLdisc™ are absolutely elec- trical, the CELLdisc™ allows storage of the single layer CELLdisc™ on top of the CD1’s CELLdisc™ production unit. To ensure suitability in users, customers are advised to test Greiner Bio-One systems under the conditions defined in their own protocols.

**Instructions for Use**

**CELLdisc™ - Multi Layer Device (1 - 40 Layers)**

**CELLdisc™ Accessories**

**CELLLevator™**

**Stacking device for CELLdisc™**

Item No. 878071

For easy and secured CELLdisc™ stacking the CELLevator™ is positioned within the circumferential rim of the top plate of the CELLdisc™. Another CELLdisc™ then be positioned on top of the CELLdisc™.

**CELLstage**

**Filling accessory for CELLdisc™**

Item No. 878072 (for 4-24 layer CELLdisc™)

878073 (for 40 layer CELLdisc™)

** CELLdisc™ Product Overview**

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Layers</th>
<th>Surface treatment</th>
<th>Surface [cm²]</th>
<th>Min. working volume [ml]</th>
<th>Max. working volume [ml]</th>
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</tr>
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</table>

* 70 ml is the maximum filling volume per layer. With more than 70 ml there is a risk of liquid flowing from one layer to the next. For cell cultivation, a working volume of 50 ml per layer is recommended.

**General CELLdisc™ Details**

For further information and accessories please visit our website or download our CELLdisc™ accessory flyer F073254 from the Download Center.

A video animation showing the handling of the CELLdisc™ is available on our website: www.gbio.com

For further information please visit our website www.gbio.com or contact us.

**General Laboratory Product for cell culture to be used by qualified personnel in a laboratory environment.**

**General CELLdisc™ Details**

The Greiner Bio-One CELLdisc™ is a ready-to-start, multi layer device, as easy to use as a T-Flask. The innovative stra- tegic, the CELLdisc™ design provides a versatile system for the propagation of adherent mammalian cells from re- search scale to industrial batches. It is available either with the standard tissue culture surface (TC; red screw cap) or the Advanced TC™ surface (blue screw cap) identical to all Greiner Bio-One cell culture products to assure consistent performance from lot to lot and from format to format.

**Intended Use**

General laboratory product for cell culture to be used by qualified personnel in a laboratory environment.

**Revision 03: June 2020 - F000360**
Instructions for Use

**Single Layer CELLDisc™**

1. Unpack the single layer CELLDisc™ and place it in a laminar air flow cabinet in order to work in sterile conditions.
2. Prepare cell suspension in accordance with the concentration (cells/cm²) used with other disposables for adherent cell culture. Unscrew screw cap and transfer the cell suspension directly into the CELLDisc™ using the large opening port by pouring or pipetting (Fig. 1). Firmly tighten the screw cap onto the CELLDisc™ to close it.
3. Tilt the single layer CELLDisc™ gently from one side to the other to assure that media and cells distribute evenly.

**Note:** To obtain equivalent cell growth in all layers, formation of air bubbles must be avoided during processing to the CELLDisc™ (processing). Therefore, an exact angle of 30° and a specific position of the central filling channel (see Fig. 2, Fig.3 and Fig.4) must be maintained during filling of 4-24 layer CELLDisc™. This handling procedure guarantees that the pressure is equalized through the central gas channel (indicated in blue in Fig. 5) without contact with the filled liquid. Thus, the air does not flow through the liquid and does not create air bubbles. Vigorous shaking of the CELLDisc™ is not recommended. Larger volumes of liquids should be mixed outside the CELLDisc™ and then added to the disposable as described below. Small amounts can be pipetted directly into the CELLDisc™ and then distributed to all layers by repeating the equalization process.

4. To simplify filling, the CELLDisc™ 4-24 layers can be positioned on the CELLevator™ (Fig. 13). More details on CELLevator™ can be found on the reverse side.

**Warning:** The media or cell suspension should not touch the filter. If the filter has absorbed any fluid, this will inhibit any gas transfer into and out of the CELLDisc™ in this case the disposable has to be discarded and a new single layer CELLDisc™ has to be used.

**CELLdisc™ 4 - 24 Layers**

1. Unpack the CELLDisc™ and place it in a laminar air flow cabinet in order to work in sterile conditions.
2. Prepare cell suspension in accordance with the concentration (cells/cm²) used with other disposables for adherent cell culture.
3. Hold the CELLDisc™ at an angle of 30° below. Small amounts can be pipetted directly into the CELLDisc™ and then distributed to all layers by repeating the equalization process.
4. Turn the CELLDisc™ as indicated in Fig. 10 to disconnect media flow from the filling channel. Do not rotate the CELLDisc™ any further as this could lead to welding of the filter.
5. From this position raise the CELLDisc™ upright (Fig. 11) and place the disposable on a horizontal surface inside an incubator (Fig. 12). Proceed with the cultivation based on the appropriate protocol.

**Note:** Small volumes of liquids (e.g. trypsin) may accumulate in the upper layers during filling and not flow through the whole filling channel. To guarantee equal distribution the liquid must be in contact with all layers through the filling channel before the equilibration process is initiated. Therefore the CELLDisc™ must be positioned horizontally with the opening port at the lowest position.

6. For liquid removal, unscrew screw cap and tilt the CELLDisc™ slowly 90° with the large opening port at the lowest possible position and pour out the media or use a pipetting system as displayed in Fig. 14 and 15.

**CELLdisc™ 40 Layers**

1. As with the CELLDisc™ 4-24 layers, air bubble formation must be avoided during processing to obtain even cell growth in all layers. While the position of the screw cap/filling channel and the general filling procedure is identical (Fig. 3/4) a smaller angle of 20° must be maintained during filling of a 40-layer CELLDisc™ (Fig. 16). This handling procedure guarantees that the pressure is equalized through the central gas channel (see Fig. 17, indicated in blue) without contact with the filled liquid. Thus, the air does not flow through the liquid and does not create air bubbles. To simplify filling, the CELLDisc™ can be positioned on the CELLevator™ filling aid (see reverse side).

2. To start liquid equilibration lay down the CELLDisc™ horizontally and turn it as displayed in figure 18 to assure that the media and all layers are in contact through the central filling channel. The media will flow equally between all the layers (Fig. 19). Turn the CELLDisc™ as indicated in Fig. 19 to disconnect media flow from the filling channel. From this position raise CELLDisc™ upright (Fig. 21) and place the disposable on a horizontal surface inside an incubator.

**Warning:** During transport, tilt the CELLDisc™ slightly backward to assure that there is no liquid contact with the filling channel or accidental media flow to another layer.

3. Liquid removal is identical to CELLDisc™ 4-24 layers (Fig. 14/15).

To simplify filling, the CELLDisc™ can be positioned on the CELLevator™ (Fig. 13). More details on CELLevator™ can be found on the reverse side.

Visit until the liquid is distributed into the individual layers still keeping the CELLDisc™ in the indicated position before firmly tighten the screw cap to close it (Fig. 6). To simplify liquid equilibration lay down the CELLDisc™ horizontally and turn it as displayed in Fig. 7/8 to assure that the media and all layers are in contact through the central filling channel. The media will now separate equally between all layers (Fig. 9).

To stack individual CELLDisc™ or a single layer CELLDisc™ on top of a production CELLDisc™ use the CELLelevator™ (Fig. 1). More details on CELLelevator™ can be found on the reverse side.