INSTRUCTIONS FOR USE

CELLDISC
Multi Layer Device
(1 - 40 Layers)
SIMPLIFY YOUR CELL CULTURE WORK PROCESS

CELLDISC MULTI LAYER DEVICE (1 - 40 LAYERS)

The Greiner Bio-One CELLdisc is a ready-to-start, multi layer device, as easy to use as a T-flask. The innovative ergonomic CELLdisc design provides a versatile system for the propagation of adherent mammalian cells from research scale to industrial batches. It is available either with the standard tissue culture surface (TC; red screw cap) or the Advanced TC surface (Adv. TC, 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 products for cell culture to be used by qualified personnel in a laboratory environment.

Figure 1: CELLdisc with 1, 16 and 40 layers

Figure 2: General CELLdisc details

CELLDISC SINGLE LAYER

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 the multilayer CELLdisc are absolutely identical, the CELLevator allows storage of the single layer CELLdisc on top of the CELLdisc production unit. To maintain the accustomed quality and efficiency of cell culture, customers are advised to test Greiner Bio-One systems under the conditions defined in their own protocols.

Integrated gas channel: Supports optimal airflow

Filter port: For pressure equilibration

Large opening port: For easy filling

Connecting channel: For liquid transfer

Rim assures equal thermal distribution
1/ 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.

3. Unscrew screw cap and transfer the cell suspension directly into the CELLDisc using the large opening port either by pouring or pipetting.

   Firmly tighten the screw cap onto the CELLDisc to close it.

4. Tilt the single layer CELLDisc gently from one side to the other to assure that media and cells distribute evenly.

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.
Unpack the CELLdisc and place it in a laminar air flow cabinet in order to work in sterile conditions.

Prepare cell suspension in accordance with the concentration (cells/cm²) used with other disposables for adherent cell culture. Hold the CELLdisc at an angle of 30°.

Hold the CELLdisc with the screw cap at a position of approximately 105° for right-handed users or 255° for left-handed ones.

To simplify filling procedure, the CELLdisc can be positioned on the CELLstage filling device (see ordering information).

**WARNING**

To obtain equivalent cell growth in all layers, formation of air bubbles must be avoided during CELLdisc processing. Therefore, an exact angle of 30° and a specific position of the connecting channel must be maintained during filling of 4-24 layer CELLdisc. This handling procedure guarantees that the pressure is equalized through the connecting channel (indicated in blue in chapter 2, step 3) without contact with the filled in liquid. Thus, the air does not flow through the liquid and does not cause foaming. In addition to the filling process, any generation of air bubbles should be avoided. 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 equilibration process.
Unscrew screw cap and add the cell suspension either by pouring or pipetting directly into the CELLdisc using the large opening port.

The media will fill the topmost layer first and then move slowly to each layer underneath.

Wait 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.

To start liquid equilibration lay down CELLdisc horizontally and turn it to assure that the media and all layers are in contact through the connecting channel.

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 CELLdisc has to be used.
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 equilibration process is initiated. Therefore the CELLdisc must be positioned horizontally with the opening port at the lowest position.

The media will now distribute equally between all layers.

Turn the CELLdisc to disconnect media flow from the filling channel.

Do not rotate the CELLdisc any further as this could lead to contact between liquid and filter.
**2/ CELLDISC 4 - 24 LAYERS**

From this position raise the CELLDisc upright and place the disposable on a horizontal surface inside an incubator.

Proceed with the cultivation based on the appropriate protocol.

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**NOTE:** 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.

To stack individual CELLDisc or a single layer CELLDisc on top of a production CELLDisc use the CELLelevator.

More details on CELLelevator can be found on the reverse side.

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.
3/ CELLDISC 40 LAYERS

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 to recommended handling of CD4-24 a smaller angle of 20° must be maintained during filling of a 40-layer CELLdisc.

This handling procedure guarantees that the pressure is equalized through the connecting channel (indicated in blue) without contact with the filled in liquid. Thus, the air does not flow through the liquid and does not create air bubbles.

To simplify filling procedure, the CELLdisc can be positioned on the CELLstage filling device (see ordering information).

To start liquid equilibration lay down the CELLdisc horizontally and turn it 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.
3/ CELLDISC 40 LAYERS

NOTE: To guarantee equal distribution, the liquid must be in contact with all layers through the connecting channel before equilibration process is initiated. Therefore the CELLDisc must be positioned horizontally with the opening port at the lowest position.

4. Turn the CELLDisc to disconnect media flow from the filling channel.

5. From this position raise CELLDisc upright and place the disposable on a horizontal surface inside an incubator.

Liquid removal is identical to the recommended handling of CELLDisc 4-24 layers.
HARVESTING OF CELLS

The protocol for harvesting cells from a multilayer device such as CELLDisc which does not allow for direct pipette 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.

This cell harvest protocol refers to standard techniques and specific suggestions to gain maximum cell yields.

In general, we recommend using the same dissociating solution and concentrations for enzymatic detachment that is used to harvest these cells from a standard Greiner Bio-One cell culture disposable. As the surface treatments and basic materials are identical for these vessels, standard protocols can be converted.

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 (trypsin, 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 approx. 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 add 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.

Incubate 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. 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 dissociation agent is desired, then spin cell suspension at 100xg for 5 minutes. Remove the dissociation agent containing media and resuspend cell pellet in fresh media.
### CELLdisc with TC surface

**Sterility SAL 10^−6**

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### CELLdisc with TC surface

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### CELL disc with external filter

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### CELL disc with closed filling caps

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### CELLdisc Accessories

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