

## Cultivation and passaging

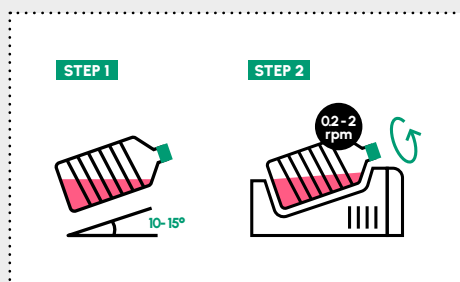
**STEP 1** Prepare a sufficient seed train several days prior to seeding the CellScrew® depending on the desired cell line and its growth rate. We recommend seeding at a slightly higher cell density to ensure a good growth rate and a viable culture. After reaching a confluency of 80 - 95 %, the cells can be processed and used to inoculate the CellScrew®.

**STEP 2** Prepare the total volume of growth medium for your CellScrew® size according to the manual and medium consumption of your cell line and prewarm it. Take the seed train flasks out of the incubator and check for contaminations, the right morphology and the confluency using a microscope. Detach the cells from the seed train flasks, wash them, and suspend them in fresh medium. Add the concentrated cell suspension to the prepared volume of growth medium to adjust the correct cell density of the inoculum. Transfer the inoculum into the CellScrew® by pouring the cell suspension from the bottle. Hold the CellScrew® at an angle and rotate it clockwise to distribute the cell suspension equally inside the inner structure.

STEP 1



STEP 2



## Expansion

**STEP 1** Remove the CellScrew® from the aseptic working bench. Hold the angle during transport to prevent the liquid to flow back to the bottom of the CellScrew®.

**STEP 2** Place the CellScrew® into an angled (10-15°) roller device located in an incubator with the desired temperature, humidity, and atmosphere. Set the roller device to a rotational speed between 0.2-2 rpm. Close the incubator and start cell expansion.

## Harvest of the CellScrew®

**STEP 1** Depending on the cell line and inoculation density, the CellScrew® is ready for harvest after 3-7 days. Take the CellScrew® out of the incubator and place it upright in an aseptic working bench. Discard the spent medium using an aspiration pipette and/or a serological pipette.

**STEP 2** Pour DPBS into the CellScrew®, hold it at an angle and turn the CellScrew® clockwise to wash the surface area and the cells attached to it. Discard the DPBS using an aspiration pipette and/or a serological pipette. Add trypsin or a detachment agent of your choice to the CellScrew®. Hold it at an angle and turn the CellScrew® clockwise to distribute the detachment agent. Place the CellScrew® into an angled (10-15°) roller device located in an incubator. Set the roller device to a rotational speed between 0.2-2 rpm. Close the incubator and incubate for the desired detachment time.

**STEP 3** Take the CellScrew® out of the incubator and place it upright in an aseptic working bench. Add FBS containing growth medium or a similar inhibitor to the detachment agent. Hold the CellScrew® at an angle and tap it while rotating it clockwise 10-15 times to rinse off still lightly attached cells. Bring the CellScrew® to an upright position and let the cell suspension flow to the bottom of the CellScrew®. Harvest the cell suspension with a 50 mL serological pipette and transfer it into a harvest bottle for further use.

STEP 1



STEP 2



STEP 3

