

Application recommendations for cold cell storage using ChillProtec®

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When kept in the new protective medium ChillProtec®, adherent cells, cell suspensions or small tissue pieces are able to remain intact after cold storage. The protective medium reduces cell damage caused by cold. Furthermore, cell functions are retained after cold storage in ChillProtec® better than after cold storage in alternative solutions. The new medium is ready for use, sterile, free of animal components, and completely chemically defined. ChillProtec® is a registered trademark and its formulation is proprietary.

ChillProtec® is suitable for the cold storage of all cell types, including primary cells. Primary human hepatocytes, for example, remained intact at 2-8 °C for several days. In addition, ChillProtec® qualifies for the temporary storage of fresh clinical specimens until cell isolation. It is also suited for the short-term storage of remaining or isolated cells, as well as for the transport of cells and tissue.

We offer two variations of ChillProtec®: ChillProtec® and ChillProtec® plus. The macromolecular substance that ChillProtec® plus contains has an additional protective effect on different cell types. You should therefore test cells using both versions. Please find below recommendations on how to use the new protective medium.

1 Overview and application principle

ChillProtec® is a medium for the cold storage of adherent cells, cell suspensions or small tissue preparations. When kept in this protective medium, cells are able to remain intact after cold storage without any loss of functionality.

Cold (hypothermia) is a widely used protection principle for the storage and transport of cells. It slows down cell metabolism and reduces damaging processes caused by lack of oxygen or substrate. It is this cold, however, that causes damages within the cells. ChillProtec® prevents such damages, allowing for significantly longer periods of cold cell storage than seen with normally used liquids, such as cell culture media, physiological salt solutions or organ protection solutions.

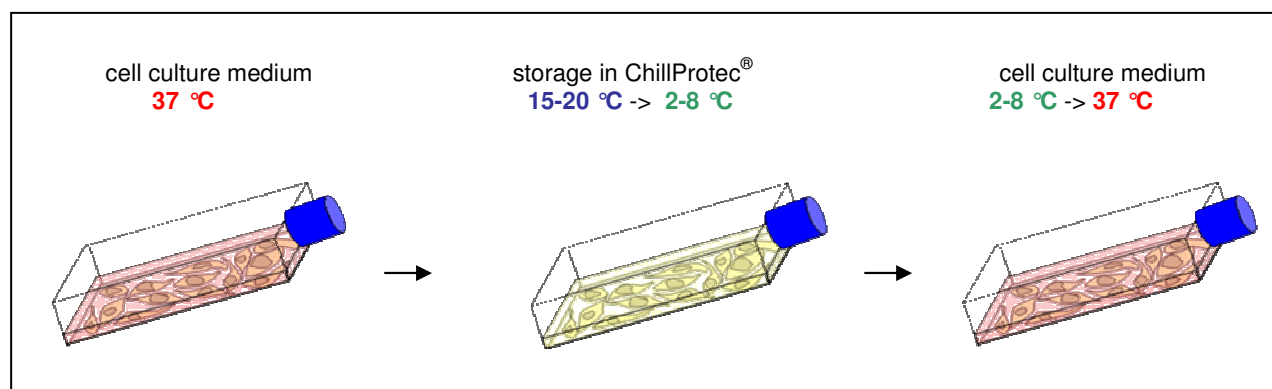


fig. 1: application principle

We offer two variations of the protective medium: ChillProtec® and ChillProtec® plus. There is a slight difference in the composition of both versions. ChillProtec® plus contains a macromolecular additive that has an additional protective effect on certain cell types.

The medium is free of animal components and completely chemically defined. The formulation of the medium is proprietary.

2 Application

2.1 General information

ChillProtec® is a sterile and ready-for-use medium for the protection against cold.

In order to avoid frequent temperature fluctuations during cell storage, it is advisable to use a less frequently used refrigerator or a cold storage room.

During the transport of cells and tissue on ice, ice water should be used in order to prevent frost damages. If ice packs are used during the transport, you should ensure that the ice packs do not have any direct contact with the cell culture flask or the vessel containing the tissue. During the transport, cell culture flasks should be completely filled with ChillProtec®.

In case of longer transports, check that the temperature is kept at 2-8 °C (range of tolerance: 0-10 °C), by means of a data logger for example.

2.2 Adherent cells

When storing adherent cells, ChillProtec® should be warmed up to 15-20 °C immediately before application.

If possible, the cells should be in their logarithmic growth phase. Approximately 24 hours before cold storage, a complete medium change should be performed. For longer periods of cold storage, cell culture flasks are the culture vessels to be preferred.

1. Slightly warm up the protective medium at room temperature (RT) to reach 15-20 °C.
2. Prior to the cold storage, wash the cells under a laminar flow with (warm) HBSS or PBS (twice or three times); exhaust HBSS or PBS respectively.
3. Put the protective medium (15-20 °C) at the cells (use the same volume that is normally used when using cell culture medium; e.g. 5 ml for a 25 cm² flask, 15-20 ml for a 75 cm² flask, 2 ml per well of a 6-well plate). When finished, close the cell culture flasks. Cover gas permeable caps with Parafilm. Store cell culture vessels in a refrigerator or a cold storage room (2-8 °C).

If you use wells or cell culture dishes, place a cap on them and mask the margins with Parafilm or adhesive tape (one strip along the entire opening or above all four lateral edges respectively). Store wells or cell culture dishes in a refrigerator or a cold storage room (2-8 °C).

5. At the end of the needed storage period, take the cells out of the refrigerator/cold storage room and use the laminar flow to exhaust the protective medium.

6. Take normal cell culture medium (complete medium with serum and further additives normally required for the respective cell type) directly out of the refrigerator (approx. 2-8 °C) and apply it to the cells. Store the cells in an incubator.

7. Change the cell culture medium the following day; in case that a larger number of cells should have detached after longer periods of cold storage, replace the medium after 4 to 6 hours after warming up.

8. Split the cell culture 48 hours after warming up if possible, but not earlier than 24 hours after warming up.

➤ **Important notes:**

Cells may contract or round off at the end of the cold storage period. This is, however, quickly reversible in most cases. If cells detach from the cell culture vessel during cold storage, you should use collagen- or fibronectin-coated cell culture vessels.

Deposits of ChillProtec® on the cells are harmless. Cells should not be washed prior to the first re-change to the cell culture medium.

2.3 Cell suspensions

Cell suspensions should be kept at 2-8 °C.

1. Pelletise cells by means of centrifugation and exhaust supernatant. Wash the cells once with PBS or HBSS.

2. Take up cells with a density of approx. 10^6 cells/ml in the protective solution (2-8 °C) and thoroughly resuspend the cells. Place the cell suspension in a cell culture dish or a sterile cell culture tube (for storage only, fill the cell culture tube to approx. 2/3; if a transport is intended, fill it completely) and close the dish or tube.

3. Store cells at 2-8 °C (tubes: place horizontally).

4. After the cold storage phase, pelletise cells by means of centrifugation and exhaust ChillProtec®.

5. Resuspend pellet in normal cell culture medium (approx. 2-8 °C), seed the cells and warm them up in an incubator or use the cells otherwise.

➤ **Important notes:**

Deposits of ChillProtec® in the medium which is used for warming up do not pose any risk. Depending on the type of application, cells may be even warmed up in the protective medium for up to 2 hours (slow warming up in an incubator). Multiple centrifugation or centrifugation at high speed after cold storage should be avoided.

2.4 Small tissue preparations

Small tissue pieces should be stored at 2-8 °C.

1. If applicable, prepare tissue preparation as desired and/or wash it with HBSS, PBS or Ringer's solution.
2. Fill tissue preparation in a sterile vessel of adequate height or in a 50 ml cell culture tube (featuring a flat bottom preferably) with cold (2-8 °C) ChillProtec®; the medium's volume should be sufficient to cover the tissue piece completely (if transport is intended, fill the vessel completely).
3. Close the vessel and store it in a refrigerator or a cold storage room (2-8 °C).
4. At the end of the cold storage period, slowly warm the tissue piece in ChillProtec® to reach room temperature (for a period of 10 to 15 minutes; for example, put the cell culture vessel into warm water featuring a temperature of 20-25°C). Place the tissue preparation into the medium intended for further use (depending on the type of application, wash with HBSS, PBS or Ringer's solution at room temperature; this is, however, not compulsory for most applications).
5. Continue using the tissue preparation as normal.

3 Suggestions on how to store cells in ChillProtec®

3.1 Medium selection

Compared to cell culture media, physiological salt solutions and organ protection solutions, ChillProtec® causes significantly less cell damage after cold storage of cells and tissue (cf. tab. 1).

tab. 1: cells and tissue tested with ChillProtec®

ChillProtec®
aortic endothelial cells (primary, porcine)
liver endothelial cells (cell line, rat)
Vero B4 (kidney epithelial cell line, monkey)
LLC-PK1 (kidney epithelial cell line, porcine)
hepatocytes (primary, human), adherent
hepatocytes (primary, human), suspension
hepatocytes (primary, rat), adherent
hepatocytes (primary, rat), suspension
hepatocytes (primary, mouse)
hepatocytes (primary, porcine)
HepG2 (hepatoma cell line, human)
A549 (lung epithelial cell line, human)
L929 (fibroblast cell line, mouse)
RIN-m5f (islet cell line, rat)
K-562 (myeloma cell line, human)
muscle (diaphragm, mouse)

As there are the two different versions of the protective medium, you should test both media. At the moment, comparative studies of ChillProtec® and ChillProtec® plus are available for a broad range of cells, recommending the use of the respective medium mentioned (cf. tab. 2).

tab. 2: cells tested with ChillProtec® and ChillProtec® plus

ChillProtec®	ChillProtec® plus
aortic endothelial cells (primary, porcine)	L929 (fibroblast cell line, mouse)
Vero B4 (kidney epithelial cell line, monkey)	hepatocytes (primary, rat)
hepatocytes (primary, human)	A549 (lung epithelial cell line, human)
liver endothelial cells (cell line, rat)	RIN-m5f (islet cell line, rat)
hepatocytes (primary, mouse)	
hepatocytes (primary, porcine)	
HepG2 (hepatoma cell line, human)	

3.2 Storage period

The possible length of cold storage highly depends on the respective cell type and cultivation or the use of the cells respectively, varying between three days and more than two weeks (up to five weeks). Users should test the storage period for each cell type and each type of application.

4 ChillProtec[®] overview

tab. 3: product details

parameter	ChillProtec [®]		ChillProtec [®] plus	
cat. no.	F 2283	F 2285	F 2293	F 2295
unit	100 ml	500 ml	100 ml	500 ml
storage	+2 - +8 °C			
raw material	chemically defined, proprietary formulation			
intended use	cold storage of cells			
note	for in vitro use only			

Order a free sample of ChillProtec[®] and ChillProtec[®] plus at: info@biochrom.de