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TECHNICAL INFORMATION

Catalog Number: 2030046, 2030026, 20300M2
CellVation Cryopreservation Medium

CellVation[tm]: An alternative serum-free cryopreservation medium

To complete the serum-free product range, MP introduces CellVation[tm], a unique cryopreservation medium which does not contain either DMSO or serum. This cell freezing medium offers the following benefits:

– No presence of serum during freeze/thaw-cycles of cells adapted to serum-free growth
– Absence of cytotoxicity or morphological differentiation associated with DMSO
– Optimal recovery and faster cell attachment to substrate
– Ready to use

CellVation[tm] has a proven track record of successful freeze/thaw cycles of over 30 cell types, including primary and finite life cells as well as suspension and anchorage-dependent cell lines.
CellVation[tm] Cryopreservation Medium is

– Completely defined - No serum
– Ready-to-use solution
– Low cytotoxicity: No DMSO (Dimethyl sulfoxide)
– Proven track record of successful freezing of > 50 cell types: cell lines and primary cells
– Completes the MP serum-free product range: cells that have been adapted to serum free growth can then be stored in serum-free environment.

The following is a brief listing of the cell types that have been successfully frozen in MP's cryopreservation medium CellVation[tm].

Cell types used with CellVation[tm]

Cell Type	Description
A-375	Malignant Melanoma, Human
A549	Lung Carcinoma, Human
D283 Med	Medulloblastoma, Human
Daoy	Medulloblastoma, Human
DU 145	Carcinoma, Prostate, Metastasis to Brain, Human
HeLa	Epitheloid Carcinoma, Cervix, Human
Hep-2	Epidermoid Carcinoma, Larynx, Human
LNCaP	Metastatic Prostate Adenocarcinoma, Human
LS 174T	Colon, Adenocarcinoma, Human
MCF7	Breast Adenocarcinoma, Pleural Effusion, Human
MCF 10	Mammary Gland, Human
MRC-5	Embryonal Lung, Diploid, Male, Human
NCI-H69	Small Cell Carcinoma, Lung, Human
PC-3	Prostate Adenocarcinoma, Human
Primary	Bone Marrow and Peripheral Blood Stem Cells, Human
Primary	Lymphocytes, Human
Raji	Burkitt's Lymphoma, Human
SF	Foreskin, Human
ST486	Burkitt's Lymphoma, Human
U-937	Histiocytic Lymphoma, Human
3T3	Embryonic Fibroblast, Mouse
B16BL6	Mouse, Melanoma
B16F10	Mouse, Melanoma
NSO	Myeloma, Mouse
P31NSI/Ag4-1(NS-1)	Non-secreting Myeloma, Mouse
Sp2/0-Ag14	Hybridoma, Non-secreting, mouse
Rat2	Embryo, Thymidine Kinase Mutant, Rat
CBA	Aorta Endothelial, Bovine (primary)
MDBK	Kidney, Bovine

CHO-K1Ovary, Chinese Hamster
hilDCKKidney, Canine
CRFKKidney, Feline
LLC-PKKidney, Porcine
STFetal Testis, Porcine (primary)
VeroKidney, African Green Monkey
ZF4Embryonic Fibroblastic Cells, Zebra Fish
JB6C1 30-7bEpidermis Tumor, Mouse
JB6C1 41-5aEpidermis Tumor, Mouse
RT 101Epidermis Tumor, Mouse
RAW 264.7Monocyte-Macrophage, Mouse
CPA-47Pulmonary Artery, Bovine
CPAEPulmonary Artery Endothelium, Bos Taurus
PU5-1.8(PU5-1R)Monocyte-Macrophage, Mouse
IC-21Macrophage, Mouse
P388DMonocyte-Macrophage, Mouse
BL-3B Lymphocytes, Leukemia Cell Suspension
BTTurbinate Cells, Bovine, Bos Taurus
CCD-18CoColon Fibroblast, Normal, Human
EBTrEmbryonic Trachea, Bovine, Bos Taurus
EJGCapillary Endothelium, Bovine
HCT 116Colon Carcinoma, Human
LB9.KKidney, Normal, Bovine
6LBLNLymph node, Ca., Bovine
LS180Colon, Adenocarcinoma, Human
293Transformed Primary Embryonal Kidney, Human

Typical Recovery Rate:

Cell Line	*% Viability before freezing*	*% Viability after freezing with CellVation*	*% Viability after freezing with 10% DMSO, 10% FBS, RPMI 1640*
SP 2/0 AG 14 Mouse Myeloma	100%	95%	90%
A 375 Human Malignant Melanoma	95%	85%	80%
U937 Human Histiocytic Lymphoma	90%	85%	60%

TCM A general purpose serum replacement2 X 10 ml2010026
produces 1 L of medium
2 X 100 ml2010022
produces 10 L of medium
TCH Does not contain animal proteins, primarily2 X 10 ml220026
for human cell cultures. Effective also onproduces 1 L of medium
other mammalian cells
2 X 100 ml2020022
produces 10 L of medium
CellVation^{tm} cryopreservation medium without20 ml2030046
serum and DMSO60 ml20300M2

Procedure for Use

CellVation^{tm} is a cryopreservation medium which does not contain DMSO or serum. CellVation^{tm} provides optimal recovery and is convenient to use. CellVation^{tm} is packaged as a 1X concentrate and should not be diluted.

Recommended Freezing and Thawing Protocol

Freezing: Mix well before use. Do not dilute.

– Examine the culture for the absence of contamination, healthy growth, confluency, etc.
– If freezing adherent cells, remove using 0.25% trypsin for 1-3 minutes at 37^{o}C. NOTE: Some cell lines grown in serum-free medium may be sensitive to 0.25% trypsin and therefore, may require less trypsin or the addition of a trypsin inhibitor.
– Perform a cell count to determine the total number of viable cells. Cell viability should be greater than 80% and cells should be in late log phase or pre-confluency growth phase.
– Centrifuge cells at 600-800 rpms for 10 minutes. Remove supernatant and save 3-5 ml for sterility testing (e.g. thioglycollate, brain heart infusion, etc.) and mycoplasma testing.
– Resuspend the cells gently in an appropriate volume of CellVation^{tm} at a concentration of 1 x 10^6 - 1 x 10^7 cells/ml. Some cell types such as hybridomas and myelomas may require an increase in cell density.
– Dispense the cell suspension in 1-2 ml aliquots in plastic or glass ampoules.
– Seal ampoules and store at room temperature for 20 - 30 minutes.
– Place ampoules in an insulated container and store in a -70^{o}C freezer for two hours. Remove insulation and transfer to the vapor phase of liquid nitrogen. If a -70^{o}C freezer is not available, place an insulated container in the vapor phase of the liquid nitrogen. Remove the insulation after two hours and store vials in vapor phase for 24 hours before transferring to liquid phase. The suggested optimum cooling rate is 1^{o}C per minute.

Recovery

– Remove vials from freezer and rapidly thaw in a 37°C waterbath.
– Wipe vials with 70% ethanol.
– Transfer cells to a culture flask and slowly add the appropriate volume of growth medium.
– Cell viability count should be performed at least 2 hours after recovery.

If desired, cells may be centrifuged in the following manner.

– Transfer cells to a 15 ml centrifuge tube and slowly add 2 - 3 mls of complete growth medium.
– Centrifuge at 400 - 600 rpms for approximately 5 minutes.
– Decant, resuspend the cells in the appropriate volume of growth medium, and transfer to culture flask.
– Cell viability count should be performed at least two hours after recovery.

IMPORTANT: Before terminating a culture, it is recommended to complete an entire freeze/thaw cycle to ensure sterility of the culture and cell viability.

Reference:

Pirro, J.P., et. al., *J. of Nuclear Medicine*, **v. 35**, #9 (Sept. 1994)

Any questions concerning MP products can be addressed directly to our technical services department: 1-800-854-0530 or 1-330-447-1200.

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