Human multipotent mesenchymal stromal/stem cells; cryopreserved primary cell culture

derived from Subcutaneous White Adipose Tissue (hADSCs)

Product:	Size:	Catalogue:	Lot:
hADSCs	1×10^6 cryopreserved cells	ADSC-000/00	000000P0

Product Description

One 2 ml-cryogenic vial, externally- or internally-threaded, labeled with cell culture information and containing 1×10^6 (±5%) ultra-low temperature frozen cultured cells resuspended in 1 to 2 ml of cryomedium, shipped on dry ice or in LN2.

Multipotent Mesenchymal Stem Cells (MSCs), also termed Multipotent Mesenchymal Stromal Cells, are self-renewing multipotent cells that can differentiate into a wide variety of cell types. It has been shown MSCs undergo *in vitro* to direct differentiation at least into three orthodoxal lineages: adipocytes, osteoblasts and chondrocytes.

The cultured MSCs originated from different human tissues are manufactured at the Vitality medical & research center biotechnology facility.

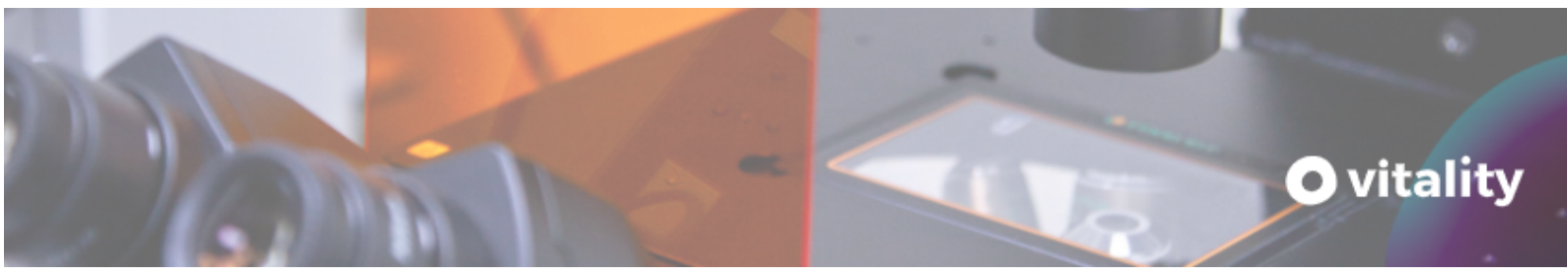
Multilineage differentiation capacity of cultured MSCs into adipocytes, osteoblasts, and chondrocytes can be done *in vitro* using, e.g., AdipoMAX (#SCM122, Sigma-Aldrich), OsteoMAX-XF (#SCM121, Sigma-Aldrich), and ChondroMAX (#SCM123, Sigma-Aldrich) Differentiation Media.

Cultured MSCs of early passages (P1-P3; passage number of the customer's choice) are cryopreserved using a defined cryomedium (see below). Each cryovial contains 1×10^6 (±5%) viable cells after thawing (no less 85% to 90% viability level by Trypan Blue dye exclusion test after shipment). Recommended cell plating density is 1–3 × 10^3 cells/cm^2.

Product Quality Control:

Rigid quality control testing is performed for each MSCs batch (both cell donors and cell cultures). Cultured MSCs are tested for cell identity, purity, potency, viability and suitability for the intended use.

Before cryopreservation cultured MSCs (P2-P3) are characterized by flow cytometry analysis for identity by a comprehensive panel of markers, namely CD73/CD90/CD105 (positive) and CD14/CD34/CD45/HLA-DR (negative) as proposed by the ISCT MSC committee position statement, 2006 [see Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315–7].



Multilineage differentiation assay into adipogenic and osteogenic directions is performed for each MSCs batch expanded *in vitro*. In addition, all donors and cell cultures have been screened for the absence of the following infectious agents: HIV-1/2, HBV, HCV, HSV1, HSV2, CMV, EBV, HHV6, *Treponema pallidum, Toxoplasma gondii, Chlamydia trachomatis, Ureaplasma urealyticum, Ureaplasma parvum* and microbial contaminants (bacteria & fungi, *Mycoplasma hominis* and *Mycoplasma genitalium*).
Cell viability after thawing or subculturing is assessed by Trypan Blue dye exclusion test (bright field microscopy; obligate) and 7-AAD fluorescent intercalator dye test (flow cytometry; optional).
MSC cultures' genetic stability is verified by karyotype evaluation (G-banding).

Intended Use:
The MSCs cultures manufactured at the Vitality research center facility are intended for R&D studies (research purposes only) and not intended for diagnostic or therapeutic application.

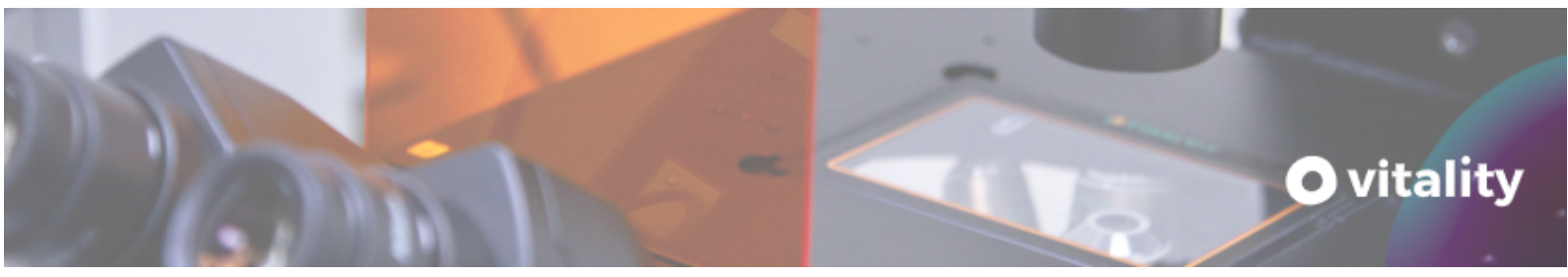
Warning:
Although tested negative for HIV-1/2, HBV, HCV, HSV1, HSV2, CMV, EBV, HHV6, *Toxoplasma gondii, Treponema pallidum, Chlamydia trachomatis, Ureaplasma urealyticum, Ureaplasma parvum* and microbial contaminants (bacteria & fungi, *Mycoplasma hominis* and *Mycoplasma genitalium*), the human cells, human cell cultures, as well as cell- and tissue-based products of human origin, should be handled as potentially infectious. Any testing procedure can't completely guarantee the absence of infectious agents in handled cell culture.
Please follow appropriate safety precautions!

Protocol for Cryopreserved Cell Culturing

Upon arrival, store/handle the cryovials with cryopreserved cells at ultra-low temperatures (e.g., liquid nitrogen, -150°C cryogenic temperature freezer) or plate them immediately over a cell culture flask

1. Prepare full growth medium and cell culture flask
Calculate the needed culture surface area according to the plating density (as a rule 1 to 3×10^3 a cm^2) according to the batch-specific cell number stated on the certificate of analysis. Fill the appropriate volume of cell culture flask, e.g., 2 × T175, 3×10^3 a cm^2 (175 cm^2 effective culturing area) with 25 ml of full growth medium per T175 flask. Place the flasks in an incubator (set at 37°C, 5% CO_2, optional 5% O_2) for 30 min for medium pre-warming up to 37°C.

2. Thaw an ultra-low temperature frozen cryovial with cells
Remove the cryovial from the dry ice/liquid nitrogen shipment container. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, and then retighten.



Immerse the cryovial into a water bath (set at 37°C), the screw cap up for 1-3 min. Ensure that no water enters the thread of the screw cap.

3. Disinfect the cryovial and plate the cells
Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the ethanol excess from the thread area of the screw cap. Unscrew the cryovial and transfer its content into the sterile 15 ml centrifuge tube, add up to 15 ml of DPBS, resuspend gently and centrifuge at 200g to 300g and RT for 5 min. Then aspirate supernatant from a tube, add 1 ml of full growth medium to cell pellet in a tube bottom and gently resuspend it. Seed the resuspended cells to a flask containing the pre-warmed full growth medium according to step 1.

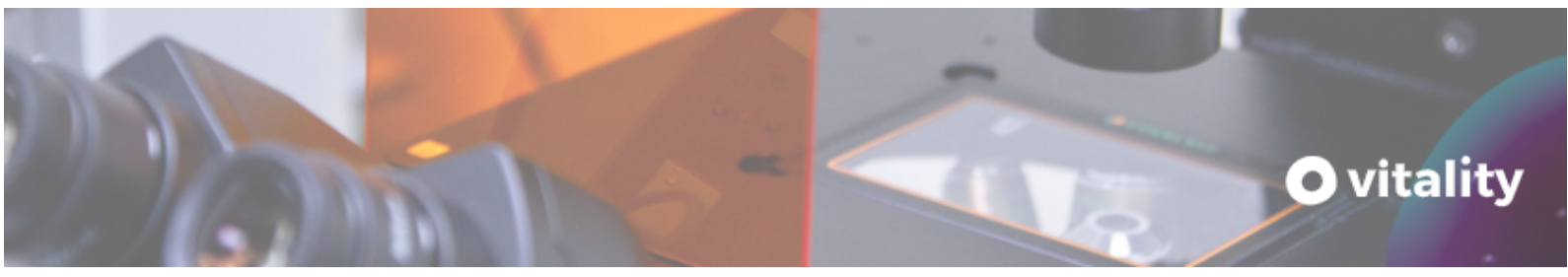
4. Incubate the cells
Place the flask in an incubator (set at 37°C, 5% CO_2, optional 5% O_2) for cell attachment. Change a medium first 24-48 hours and every two to three days thereafter. The cells should be subcultured according to the subcultivation protocol once they have reached 70 – 90% confluency. Check up the cell culture confluency level using an inverted microscope.

MSCs Subcultivation Protocol

1. Prepare the appropriate reagents and rinse the cell culture
Place the DPBS in a water bath (set at 37°C) for at least 30 min. Carefully aspirate the full culture medium from a flask with cell monolayer. Rinse a flask with pre-warmed DPBS shaking carefully for a few seconds.

2. Remove the cells from a flask
Carefully aspirate DPBS from a culture flask. Add 3-5 ml of pre-warmed (37°C) 0.25% trypsin-EDTA working solution to a flask, shake gently 1-2 times the solution over cell monolayer, then aspirate Trypsin-EDTA solution and let incubate cell monolayer in 'trypsin-EDTA vapors' for 3-5 min at 37°C in an incubator. Check up the cell detachment process with an inverted microscope. After cell detachment, add to the flask with removed cells by 3 ml of full growth medium for trypsin inactivation, shake a flask gently 1-2 times over the flask surface.

3. Cell Harvesting
Carefully aspirate the cell suspension and transfer it to a 15 ml centrifugation tube. Spin down the cells for 5 min at 200g to 300g and RT.



4. Incubate the cells

After centrifugation discard the supernatant from a tube; add 1 ml of full growth medium to the cell pellet in a tube bottom and resuspend the pellet by gently pipetting up and down. Plate the cell suspension according to the recommended plating density in a new flask containing pre-warmed full growth medium. Place the flask in an incubator (set at 37°C, 5% CO_2, optional 5% O_2) and change the medium every two to three days.

Examples of used media and solutions:

Full growth medium: basal MEM, Alpha Modification without ribonucleosides and deoxyribonucleosides (e.g., #M4526, Sigma-Aldrich), 10% FBS (e.g., #F7524, Sigma-Aldrich), 2 ng/ml hr-bFGF, 2 mM stable L-glutamine (e.g., #STA-B, Capricorn), antibiotic/antimycotic working solution: 100 units/ml penicillin, 0.1 mg/ml streptomycin, 0.25 µg/ml amphotericin B (e.g., #AAS-B, Capricorn);

Cell rinsing solution: DPBS w/o Ca2+, Mg2+ (e.g., #PBS-1A, Capricorn), antibiotic/antimycotic working solution: 100 units/ml penicillin, 0.1 mg/ml streptomycin, 0.25 µg/ml amphotericin B (e.g., #AAS-B, Capricorn);

Adherent cell removing solution: Trypsin-EDTA solution (e.g., #T4049, Sigma-Aldrich);
Cryomedium: FBS (e.g., #F7524, Sigma-Aldrich), 10% DMSO (e.g., #D2438, Sigma-Aldrich).