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hMSC chondrogenesis induction medium, serum-free kit

Cat.-Nr.: 213 0902

contains of:

Basal media		Supplements	
200 0902	500 ml hMSC chondrogenesis induction medium, basal (+4°C)	**1x** 221 1000	HEPES (-20°C)
		10x 238 0902	ITS (+4°C)
		10x 238 0903	Dexamethasone (-20°C)
		10x 238 0904	TGF-ß-3 (-20°C)
		10x 238 0905	Sodium pyruvate (-20°C)
		10x 238 0906	Asorbic-acid-2-phosphate (-20°C)
		10x 238 0907	Proline (-20°C)
		1x 236 0350	Antibiotics (optional) (-20°C)

Maintenance of hMSC chondrogenesis induction medium:

Place the bottle of **medium** in the dark at **4°C to 8°C** immediately after delivery. Store the separate delivered **Supplements at -20°C /+4°C. Take care on the instructions on the Supplement vials!!!**

Characteristics:

The Provitro hMSC chondrogenesis induction medium is a sterile liquid culture medium for inducing chondrogenic differentiation of human mesenchymal stem cells (hMSC). The medium is delivered as a basal medium and is suitable for culturing hMSC after adding all the supplement mix components. The formulation is optimized for initial seeding of 6,000 cells / cm² up to confluence (approx. 90 %). Feeder-layer, matrix substrates or other substances are not necessary. **Due to the possibility of reduced proliferative activity we recommend to use the antibiotic supplement for freshly isolated cells only.**

Stability and storage:

After adding 10 ml HEPES and optional 3,5ml antibiotics to the hMSC chondrogenesis induction medium basal the media can be stored in the dark at 4°C to 8°C for up to 4 weeks. After adding the induction supplement components 1, 2 and 3 to the media, the media can be stored in the dark at 4°C to 8°C for up to 1 week. Therefore each aliquot contains enough volume to prepare 50 ml hMSC chondrogenesis induction media only. **To prepare 50 ml chondrogenesis inductions media add 500 µl ITS, 500 µl Dexamethasone, 500 µl Sodium pyruvate, 500 µl Asorbic-acid-2-phospahte and 500 µl Proline to 50 ml hMSC chondrogenesis induction medium, basal (already supplemented with HEPES). 500 µl TGF-ß-3 must be added always fresh (see recommended application). Do not use the supplemented media for longer than one week (without TGF-ß-3).** Do not heat the medium over 37°C or use uncontrollable sources of heat (e.g. microwave appliances). If only a part of the medium is to be used, remove this amount from the bottle and heat it.

Special note:

Do not freeze the medium. This can lead to high salt concentrations by freezing out pure water which will cause irreversible damage.

Quality control:

Provitro's hMSC chondrogenesis induction medium is thoroughly tested after each production. All components are tested in a stringent biological assay. Each batch is checked for hMSC chondrogenesis induction characteristics. The cells cultured in hSMC chondrogenesis induction medium are checked regarding their morphology, the adherence rate, the colony forming efficiency and the population doubling time.

Product specification:

The pH is set at 7.6 and osmolality at 285 ± 10 mOsm / kg.

In vitro laboratory use only.

Not intended for any human or animal diagnostic or therapeutic use.

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Recommended application of hMSC chondrogenesis induction medium

Completion of culture medium:

- First of all add 10 ml HEPES and optional 3.5 ml of antibiotics to 500 ml of basal media. Having added HEPES and antibiotics, the medium can be stored in the dark at 4°C to 8°C for up to 4 weeks.

1st option: Preparing 50 ml of chondrogenesis control media (**CCM**):

- Add to 50 ml basal media (being already supplemented with HEPES) one vial of ITS (500 µl), one vial of Dexamethasone (500 µl), one vial of Sodium pyruvate (500µl), one vial of Asorbic-acid-2-phosphate (500µl) and one vial of Proline (500 µl).

NOTE: Store the prepared chondrogenesis control media at 4°C to 8°C in the dark and do not use longer than 1 week!

2nd option: Preparing 50 ml of chondrogenesis induction media (**CIM**):

- Add one vial of TGF-ß-3 (500 µl) to 50 ml of the control media (**CCM**) prepared before.

NOTE: Prepare the chondrogenesis induction medium (CIM) always fresh and use it within 12 hours! If you need less than 50 ml of chondrogenesis induction media (CIM) at one time, you may aliquot smaller volumes of chondrogenesis induction supplement 3. In general, one needs 10 µl of chondrogenesis induction supplement 3 to prepare 1 ml of chondrogenesis inductions media (CIM).

Culture protocol:

- First of all, estimate the total number of pellet cultures needed for your experiment. BEWARE: You will need $2.5*10^5$ cells to form one chondrocyte pellet. You should always work in duplicates and maybe carry a negative control (using chondrogenesis control media instead of chondrogenesis induction media during the following culturing steps).
- After harvesting your precultured hMSC, transfer for each pellet $2.5*10^5$ cells into a separate 15 ml polypropylene tube.
- Centrifuge the cells at 150xg for 5 minutes and discard the supernatant. Resuspend the cells in **500 µl of chondrogenesis control (CCM) or inductions media (CIM).**
- Centrifuge the cells again at 150xg for 5 minutes and DO NOT aspirate the supernatant, and DO NOT resuspend the pellet!
- Loosen the cap of the tubes one half turn to allow gas exchange and incubate the tubes at 37°C, 5% CO_2 for 48 hours
- Meanwhile, the cells should have formed a spherical aggregate detached from the tube wall.
- Replace every 2-3 days 90% of the supernatant with 450 µl of the corresponding medium (CCM or CIM).
- Having replaced the media, gently flick the bottom of each tube to ensure the pellet is free floating, and do not forget to loosen the gap again before returning the tubes to the 37°C incubator
- Chondrogenic pellets and also the control pellets should be harvested after 28 days in culture.
- For frozen sectioning the pellets may be embedded in Tissue Tek. Thin sections of pellet cultures may be stained e.g. with markers specific for glycoproteins