Provitro AG | Charitéplatz 1 | tel +49.30.450 578 358 | sales@provitro.de
Charité Campus Mitte | 10117 Berlin | fax +49.30.450 578 919 | www.provitro.de

hMSC osteogenesis induction medium, FCS-kit

Cat.-Nr.: 211 0903

contains of:

Basal media (+4°C)		Supplements (-20°C)	
200 0903	500 ml hMSC osteogenesis induction medium, basal	**1x** 231 5000	FCS (foetal calf serum)
		1x 221 1000	HEPES
		1x 222 1001	L-Glutamine
		10x 238 0903	Dexamethasone
		10x 238 0908	Ascorbic-Acid-2-phosphate
		10x 238 0909	ß-Glycerol-phosphate
		1x 236 0350	Antibiotics (optional)

Maintenance of hMSC osteogenesis induction medium:

Place the bottle of **medium** in the dark at **4°C to 8°C** immediately after delivery. Store the delivered **Supplements at -20°C**.

Characteristics:

The Provitro hMSC osteogenesis induction medium is a sterile liquid culture medium for inducing osteogenic differentiation of human mesenchymal stem cells (hMSC). The medium is delivered as a basal medium and is suitable for culturing hMSC after adding 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.**

Reconstitution, stability and storage:

After adding 50 ml FCS, 10 ml HEPES and 5 ml L-glutamine and optional 3,5 ml Antibiotics to the 500 ml hMSC osteogenesis induction medium basal the media can be stored in the dark at 4°C to 8°C for up to 4 weeks. **Take care:** After adding the Osteogenesis induction factor Dexamethasone, Ascorbic-Acid-2-phosphate and ß-Glycerol-phosphate 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 osteogenesis induction media only. To prepare 50 ml osteogenesis inductions media add 500 µl Dexamethasone, 500 µ Ascorbic-Acid-2-phosphate and 500 µ ß-Glycerol-phosphate to 50 ml hMSC osteogenesis induction medium, basal (already supplemented with FCS, HEPES and L-Glutamine). Do not use the supplemented media for longer than one week. 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 osteogenesis induction medium is thoroughly tested after each production. All components are tested in a stringent biological assay. Each batch is checked for hMSC osteogenesis induction characteristics. The cells cultured in hSMC osteogenesis 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.

Provitro AG
Charité Campus Mitte
Charitéplatz 1
10117 Berlin
tel +49.30.450 578 358
fax +49.30.450 578 919
sales@provitro.de
www.provitro.de

Recommended application of hMSC osteogenesis induction medium

Completion of culture medium:

- First of all add 50 ml FCS, 10 ml HEPES, 5 ml L-Glutamine and optional 3.5 ml of antibiotics to 500 ml basal medium. Having added those supplements, the medium can be stored in the dark at 4°C to 8°C for up to 4 weeks.

Preparing 50 ml of osteogenesis induction medium:

- Add one vial of Dexamethasone (500 µl), one vial Ascorbic-Acid-2-phosphate (500 µl) and one vial ß-Glycerol-phosphate (500 µl) to 50 ml basal media (the latter being already supplemented with HEPES, FCS, L-Glutamine).

NOTE: Store the prepared osteogenesis induction medium at 4°C to 8°C in the dark, and do not use longer than 1 week!

Culture protocol:

- After harvesting your pre-cultured hMSC, plate 5,000 cells/cm² in a 6 well-plate (1 well ~ 10 cm² = $5*10^4$ cells per well).
- Feed the cells every 2-3 days with hMSC proliferation media (e.g. provitro 201 0901) until the culture reaches 100 % confluence (approx. 5-7 days).
- Having 100% confluent hMSC culture change the media all 2-3 days with osteogenesis induction media and incubate the cells always at 37°C, 5 % CO_2.
- Possible negative controls will be fed always with osteogenesis induction media without the osteogenesis induction factor (that means basal media only supplemented with HEPES, FCS and L-Glutamine).
- After at least 28 days of culturing osteogenic structures can be detected e.g. with Von Kossa or Alkaline phosphatase staining.