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hMSC adipogenesis induction medium, FCS-kit

Cat.-Nr.: 211 0904

contains of:

Basal media		Supplements		
200 0904	500 ml hMSC adipgenesis induction medium, basal (+4°C)	**1x**	231 5000	FCS (foetal calf serum) (-20°C)
		1x	221 1000	HEPES (-20°C)
		1x	222 1001	L-Glutamine (-20°C)
		10x	225 0904	Dexamethasone (-20°C)
		10x	229 0904	Indomethacine (-20°C)
		10x	230 0904	3-Isobutyl-1-methyl-xanthine (-20°C)
		10x	246 0904	Insulin (-20°C)
		1x	236 0350	Antibiotics (optional) (-20°C)

Maintenance of hMSC adipogenesis 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**.

Characteristics:

The Provitro hMSC adipogenesis induction medium is a sterile liquid culture medium for inducing adipogenic 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 kit 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 50 ml FCS, 10 ml HEPES and 5 ml L-Glutamine and optional 3,5 ml antibiotics to the 500 ml hMSC adipogenesis 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 factor Dexamethasone, Indomethacine, 3-Isobutyl-1-methyl-xanthine and insulin 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 adipogenesis induction media only. **To prepare 50 ml adipogenesis inductions media add 500 µl Dexamethasone, 500 µl Indomethacine, 500 µl 3-Isobutyl-1-methyl-xanthine and 500 µl Insulin to 50 mL hMSC adipogenesis 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 adipogenesis induction medium is thoroughly tested after each production. All components are tested in a stringent biological assay. Each batch is checked for hMSC adipogenesis induction characteristics. The cells cultured in hSMC adipogenesis 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 adipogenesis 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.

1st option: Preparing 50 ml of adipogenesis maintenance medium (**AMM**):

- Add one vial of Insulin (500 µl) to 50 ml basal media (the latter being already supplemented with HEPES, FCS, L-Glutamine).

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

2nd option: Preparing 50 ml of adipogenesis induction medium (**AIM**):

- Add one vial of Insulin (500 µl), one vial of Dexamethasone (500 µl), one vial of Indomethacine (500 µl) and one vial of 3-Isobutyl-1-methyl-xanthine (500 µl) to 50 ml of basal medium (the latter being already supplemented with HEPES, FCS, L-Glutamine).

NOTE: Store the prepared adipogenesis inductions media 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, 3 cycles of inductions and maintenance follow:

Precultrue	1st cycle		2nd cycle		3rd cycle	
until 100% confluence	AIM 3 days	AMM 2 days	AIM 3 days	AMM 2 days	AIM 3 days	AMM 2 days

- Change the media all 3 or 2 days according to the above scheme with fresh adipogenesis induction media (**AIM**) or adipogenesis maintenance media (**AMM**), and incubate the cells always at 37°C, 5 % CO_2.
- Possible negative controls will be fed always with adipogenesis maintenance media (AMM).
- After 15 days of culturing the cells should show lipid vacuoles which can be detected e.g. with Oil Red O.