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ORION

Product Description

StemGold MSC Basal Medium is a serum-free, xeno –free medium formulation developed for the growth and expansion of human mesenchymal stem cells after being isolated from a variety of sources (ie bone marrow, adipose tissue and umbilical cord tissue; BM-hMSC. AT-hMSC, UCT-hMSC).

For initial isolation of hMSC, we recommend 2.5% human AB serum to be added to facilitate cell attachment and growth.

StemGold MSC Basal medium supports long term growth of hMSC while maintaining their self renewal and multi-lineage differentiation potential.

No adaptation is required for the transition from serum-containing medium to Stem Gold MSC medium.

Notes:

- No additional additives are required for the complete , ready to use medium
- Contains L-glutamine
- Doesn't contain antibiotics

Features

- Serum free, xeno free. All components are defined and from non-xenogenic origin including proteins
- Enables culture of hMSC from different sources
- Supports long term growth of hMSC retaining the fibroblast like cell culture
- No background differentiation
- Maintains hMSC self renewal and multi-lineage differentiation potential (eg osteocytes, adipocytes and chondrocytes)
- Human MSC cultured Stem Gold express high percentage of MSC surface markers and do not express hematopoietic markers

Adaptation of hMSC to StemGold

hMSC can be transferred directly without prior adaptation.

Complete Ready To Use Medium Preparation

The frozen Stem Gold supplement Mix should be thawed at room temp or at 2-8 Deg . Avoid Repeated freeze thaw cycles

For a complete medium, aseptically add 0.6ml of Stem Gold supplement Mix to 100ml of StemGold Basal medium.

(Alternatively add 3ml of StemGold Supplement Mix to 500ml of StemGold Basal medium).

Culturing of hMSC in the complete Stem Gold Basal medium

Recovery of Cryopreserved hMSC

- Prewarm 5-10ml of complete StemGold Medium in a 50ml conical tube
- Rapidly thaw frozen vial of hMSC in a 37Deg waterbath with agitation until a small amount of ice remains
- Slowly add (drop by drop while gently swirling) the cells into the pre warmed complete Stem Gold medium
- Centrifuge cells at 300-400xg for 4-5 mins at room temp
- Remove supernatant and re-suspend cell pellet in a 0.5-1ml of complete Stem Gold medium.
- Perform a viable cell count
- Add desired volume of complete Stem Gold medium
- Transfer cells into coated ware
- Incubate

Subculturing hMSC

StemGold Medium was developed for optimal proliferation of hMSC from a variety of sources (BM-hMSC, AT-hMSC, UCT-hMSC).

The variety sources and the variability of donors may influence hMSC proliferation rate. For optimal proliferation of hMSC in StemGold Medium, it is recommended to seed hMSC at concentration of 5000-6000 per cell/cm², refeed cells with fresh warmed complete StemGold Medium every 2-3 days and subculture when the cells reach up to 80% confluence.

Subculturing protocol

- Pre warm recombinant trypsin to room temperature
- Remove culture medium and wash gently with PBS
- For T25 culture flask add 1 -3ml of recombinant trypsin solution. Note: The more the culture is confluence, the slower the detachment will be and higher volume is needed.
- Incubate for 2 to 10 mins at room temperature and verify cell detachment using inverted microscope (incubate at 37 °C will not accelerate detachment). Usually within 2-5mins at RT the cells will dissociate by gently tapping the flask
- Following detachment add 5-10ml of pre-warmed StemGold Media.

- Centrifuge cells for 4-5mins at 300-400xg at room temperature. Discard supernatant carefully
- Re-suspend cell pellet in minimal volume of pre-warmed complete StemGold Medium. Take sample to perform cell count.
- Re-seed cells in coated well and the required volume of StemGold Medium should be added.

Table 2. Recommended seeding densities (approximately 5000-6000 cells/cm²)

| <u>Cultureware</u> | <u>12well</u> | <u>6 well</u> | <u>T25 Flask</u> |
|------------------------------|---------------|---------------|------------------|
| Surface area cm ² | 3.9 | 9.6 | 25 |
| Volume of Medium | 1-2ml/well | 2-3ml/well | 5-6ml/T-25 |

Recommended seeding densities

| <u>12well</u> | <u>6 well</u> | <u>T25 Flask</u> |
|--|--|--------------------------------------|
| 1.8-2.3 x 10 ⁴ cells / well | 4.5-5.5 x 10 ⁴ cells / well | 12-15 x 10 ⁴ cells / T-25 |