

## Instruction manual

### Osteoblast Growth Medium

Product	Size	Catalog number
Osteoblast Growth Medium (Ready-to-use) – Includes Basal Medium and SupplementMix	500 ml	C-27001
Osteoblast Basal Medium	500 ml	C-27010
Osteoblast Basal Medium, phenol red-free	500 ml	C-27015
Osteoblast Growth Medium SupplementMix	for 500 ml	C-39615

### Osteoblast Mineralization Medium

Product	Size	Catalog number
Osteoblast Mineralization Medium (Ready- to-use) – Includes Basal Medium and SupplementMix	100 ml	C-27020

#### Recommended for

- Human Osteoblasts (HOB)

#### Product description

Our Osteoblast Growth Medium has been optimized for the *in vitro* cultivation of human osteoblasts.

Our Osteoblast Mineralization Medium has been developed for the efficient induction of strong mineralization in human osteoblast cultures. Extensive extracellular calcium-deposits can be observed after 21 days by following an easy one-step protocol.

The Media are optimized for primary human cells but can also be used for bovine, murine, and rat osteoblasts as well as osteoblast cell lines.

The Media (Ready-to-use) consist of one bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix

to the Basal Medium results in the complete Medium.

Basal Medium (with or without phenol red) as well as the Osteoblast Growth Medium SupplementMix can also be purchased separately.

#### Supplementation details

The Osteoblast Growth Medium contains all the supplements necessary for the optimal growth of human osteoblasts.

The Osteoblast Mineralization Medium contains all the growth factors and supplements necessary for mineralization of human osteoblasts.

The Osteoblast Media do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO<sub>2</sub>.

#### Preparation of the supplemented medium for use

Thaw the SupplementMix at 15–25°C. Aseptically mix the SupplementMix by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

#### Instruction for use of Osteoblast Mineralization Medium

Plate  $3 \times 10^4$  HOB per well on a collagen I coated 24-well tissue culture plate (Corning, Cat. No. #354408). Work in duplicate. Use HOB Growth Medium (C-27001) for one well as a negative control and Osteoblast Mineralization Medium (C-27020) for the other well. Incubate the cells for 17–21 days. Change the medium every third day. Be careful not to disturb the cell monolayer. Fix and stain the cells for osteogenic markers.

### Storage and stability

Store the Basal Medium at 4–8°C in the dark and the SupplementMix at -20°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the SupplementMix to the Basal Medium, the shelf life of the complete medium is 6 weeks at 4–8°C. Do not freeze the complete medium.

For use, prewarm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4–8°C.

### Quality control

All lots of PromoCell Osteoblast Growth Medium are subjected to comprehensive quality control tests using primary human osteoblasts. Each lot is checked for growth promoting activity, adherence rate, and typical morphology of the tested osteoblasts. Further, all lots of PromoCell Osteoblast Mineralization Medium are tested for their capacity to induce mineralization in primary human osteoblasts. Approved in-house lots of media are used as a reference.

In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

### Intended use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

**Note:** Due to their low serum content or the absence of serum, PromoCell media are not suitable for trypsin neutralization (e.g. when splitting the cells). Instead we recommend using our DetachKit (C-41200, C-41210, C-41220), which contains HEPES BSS, Trypsin/EDTA and Trypsin Neutralizing Solution.

Final supplement concentrations (after addition to the medium)	Osteoblast Growth Medium
Fetal Calf Serum	0.1 ml/ml

---

If you require special media modifications, we offer a custom media service starting at 10 bottles per order. Contact us at [info@promocell.com](mailto:info@promocell.com) to find out more.

---

#### PromoCell GmbH

Sickingenstr. 63/65  
69126 Heidelberg  
Germany

#### USA/Canada

Phone: 1 – 866 – 251 – 2860 (toll free)  
Fax: 1 – 866 – 827 – 9219 (toll free)

#### Deutschland

Telefon: 0800 – 776 66 23 (gebührenfrei)  
Fax: 0800 – 100 83 06 (gebührenfrei)

#### France

Téléphone: 0800 – 90 93 32 (ligne verte)  
Téléfax: 0800 – 90 27 36 (ligne verte)

#### United Kingdom

Phone: 0800 96 03 33 (toll free)  
Fax: 0800 169 85 54 (toll free)

#### Other Countries

Phone: +49 6221 – 649 34 0  
Fax: +49 6221 – 649 34 40