

## Instruction Manual

Product	Size	Catalog Number
Keratinocyte Growth Medium 2 (Ready-to-use) – Includes Basal Medium, SupplementMix and CaCl <sub>2</sub> -Solution	500 ml	C-20011
Keratinocyte Growth Medium 2 Kit – Includes Basal Medium, SupplementPack and CaCl <sub>2</sub> -Solution	500 ml	C-20111
Keratinocyte Basal Medium 2	500 ml	C-20211
Keratinocyte Basal Medium 2, phenol red-free	500 ml	C-20216
Keratinocyte Growth Medium 2 SupplementMix	for 500 ml	C-39016
Keratinocyte Growth Medium 2 SupplementPack	for 500 ml	C-39011

### Recommended for

- Normal Human Epidermal Keratinocytes (NHEK), juvenile foreskin
- Normal Human Epidermal Keratinocytes (NHEK), adult donor

### Product Description

Our Keratinocyte Growth Medium 2 is a serum-free medium for the in vitro cultivation of epidermal keratinocytes without feeder cells. The medium is optimized for primary human cells, but can also be used for horse, porcine, as well as mouse and rat keratinocytes when the calcium chloride (CaCl<sub>2</sub>) concentration is adapted. In addition, it is suitable for rat corneal keratinocytes.

Keratinocyte Growth Medium 2 is available as Medium (Ready-to-use) or as Medium Kit.

The Medium (Ready-to-use) consists of a 500 ml bottle of Basal Medium, one vial of CaCl<sub>2</sub> stock solution, and one vial of SupplementMix. The Medium Kit consists of a 500 ml bottle of Basal Medium and the SupplementPack (a set of individual vials with pre-measured supplements) allowing the user full control over the media formulation.

Adding the Supplement solutions to the Basal Medium results in the complete Growth Medium.

Basal Medium (with or without phenol red), as well as SupplementMix and SupplementPack can also be purchased separately.

### Supplementation Details

Our Keratinocyte Growth Medium 2 contains

all the growth factors and supplements necessary for the optimal growth of human keratinocytes, e.g. Bovine Pituitary Extract, Epidermal Growth Factor, Insulin, and Transferrin (for details see the table on page 2). The Keratinocyte Growth Medium 2 does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO<sub>2</sub>.

**Note:** CaCl<sub>2</sub> is provided as separate stock solution. This allows users to adjust the CaCl<sub>2</sub> concentration individually. To ensure the maximal growth performance of normal human keratinocytes, we recommend adding the entire 60 µl CaCl<sub>2</sub> stock solution, which results in final 0.06 mM CaCl<sub>2</sub> concentration. In case you need a higher CaCl<sub>2</sub> concentration (e.g. for differentiation experiments), an additional CaCl<sub>2</sub>-solution must be added separately.

### Preparation of the Supplemented Medium for Use

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

### Storage and Stability

Store the Basal Medium at 4–8°C in the dark and SupplementMix, the CaCl<sub>2</sub> solution, or the SupplementPack 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 supplements 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, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4–8°C.

### Quality Control

All lots of PromoCell Keratinocyte Growth Medium 2 are subjected to comprehensive quality control tests using primary human keratinocytes. Each lot is checked for growth promoting activity, adherence rate, and typical morphology of the tested keratinocytes. Approved inhouse 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 the 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)	Size
Bovine Pituitary Extract	0.004 ml / ml
Epidermal Growth Factor (recombinant human)	0.125 ng / ml
Insulin (recombinant human)	5 µg / ml
Hydrocortisone	0.33 µg / ml
Epinephrine	0.39 µg / ml
Transferrin (recombinant human)	10 µg / ml
CaCl <sub>2</sub>	0.06 mM

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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.

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