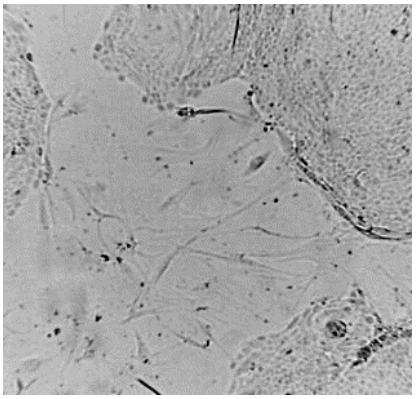
E-Mail: Info@Phenion.com

Web: www.phenion.com

Phenion® Feeder Cell Supported Keratinocyte Cultures



Preface

Albeit cell cultures with keratinocytes only are possible and often performed using the Phenion® Keratinocyte Culture Medium (K CM-250), we recommend for optimal cell culture results to conduct co-cultures with growth inhibited feeder cells. We provide cryopreserved human Feeder Cells (hFeeder), derived from juvenile male foreskin fibroblasts, which are growth-inhibited using Mitomycin C. The successful growth arrest is controlled and documented for each feeder cell batch before product release and shipment.

To achieve best results, we recommend to co-culture Phenion® Human juvenile P1 Keratinocytes with Phenion® Human Feeder Cells in K CM-250 medium in a ratio of 1:1.

The following protocol describes the procedure for setting-up the co-culture. Please note that all steps shall be conducted respecting the standards of sterile working conditions for culturing eucaryotic cells at 37°C, 5% CO_2 and ≥90% relative humidity.

Materials

Material	Supplier	Order-No.
Keratinocyte medium	phenion.com	K CM-250
Human Feeder Cells ($1*10^6$ cells in 1 ml)	phenion.com	hFeeder
Human P1 Keratinocytes ($1*10^6$ cells in 1 ml)	phenion.com	hK P1
DMSO	Sigma-Aldrich	D2650
Cell culture flasks (175 cm^2)	Greiner Bio One	660175
PBS without Ca^{2+} and Mg^{2+}	Fischer Scientific	14190169
Trypsin-EDTA	Fischer Scientific	25300096
Centrifuge tubes (15 ml), sterile	Greiner Bio One	188271

Procedure

Preparation of feeder cell culture

1. Fill 15 ml of pre-warmed Keratinocyte Culture Medium into T175 cell culture flasks.
2. Thaw a vial of feeder cells using a small volume of Keratinocyte Culture Medium (K CM-250), and carefully mix the resulting cell suspension in order to distribute the cells evenly. Seed the cells with a density of $5.0*10^5$ into each T175 flask.
3. Adjust final Keratinocyte Culture Medium volume to 25 ml for each flask.
4. Incubate for 2 to 4 days at standard cell culture conditions.

Set-up of feeder cell- supported keratinocyte culture

5. Thaw human keratinocytes (hK P1) in a small volume pre-warmed keratinocyte medium (K CM-250) and resuspend them to evenly distribute the cells. Seed the cells with a density of $5.0*10^5$ into the T175 flasks with feeder- conditioned medium. Do not aspirate the feeder cell- conditioned medium.
6. Exchange the medium every 2nd day.

   Note: Do not overgrow cells to avoid contact inhibition.

Harvesting Keratinocytes

7. Aspirate cell culture medium and wash cells once with 10 ml PBS w/o Ca^{2+}/Mg^{2+} equilibrated to room temperature before.
8. To first detach and remove the feeder cells, add 5 ml pre-warmed trypsin-EDTA solution for approx. 2 min at room temperature. Follow the process at the microscope, aspirate and discard the liquid when feeder cells are completely detached. You might support detaching by tapping the flasks slightly.
9. Add fresh 5 ml trypsin-EDTA and incubate for 5 - 7 min at 37°C to collect the keratinocytes. Enforce the detachment of cells by slightly knocking at the side of the cell culture flask.
10. Inhibit trypsin-EDTA by adding 5 ml keratinocyte medium and carefully pipet the solution up and down to dissolve potentially remaining cell aggregates. Ensure that process of enzymatic treatment is finalized within a maximum of 10 min.
11. Determine the cell yield by counting cells in e.g. a Neubauer chamber following the manufacturers manual.