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COMPETENCE IN SKIN PHYSIOLOGY

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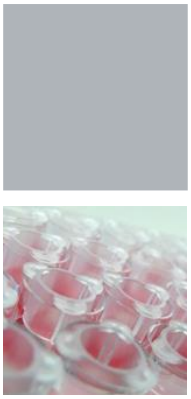
Phenion® FT Skin Model

Separation of Epidermis from Dermis



Preface

The separation of the epidermis from the dermis is mandatory for the validated 3D Skin Comet assay (see separate protocol). This method provides reliable data on genotoxicity that are required e.g. for the safety assessments or REACh registrations of chemicals.

In addition, the Phenion® FT Skin model is ideally suited to study epidermal – dermal interactions. The model allows analyses of the complete tissue as well as of each compartment, dermis and epidermis, separately.

In these cases, the following protocol guides through the separating process, allowing researchers to individually assess the epidermis and dermis – e.g. for DNA, RNA or protein examinations.

Materials/Disposables/Reagents

Material	Company	Order no.
Petri dishes, 100x20 mm	e.g. Greiner Bio-One	664160
12-well plates	e.g. Greiner Bio-One	665180
Disposable scalpel	e.g. Braun (Tuttlingen, Germany)	5518083
Bent forceps	e.g. Carl Roth	YK34.1
Thermolysin	Sigma-Aldrich	T7902
PBS without Ca^{2+}/Mg^{2+}	Fisher Scientific (Germany)	14190169

Thermolysin solution

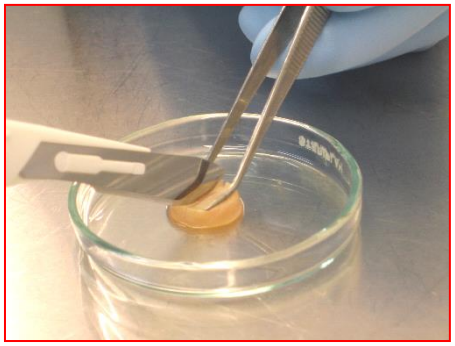
Reagent	Weight / Volume	Company	Order no.
KCl	0.5 g	Roth	6781.1
NaCl	0.58 g	Roth	9265.2
$CaCl_2$	0.147 g	Sigma-Aldrich	C5670
1 M Hepes	2 ml	Invitrogen	15630106
	up to 200 ml with H_2O_{demin}		

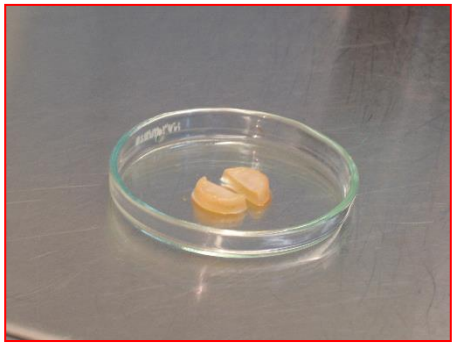
Dissolve 0.1 g Thermolysin in the buffer to final concentration of (0.5 mg/ml).
Store aliquots at -20 °C for up to 12 months.

Procedure

1. Briefly wash skin model with PBS w/o Ca^{2+}/Mg^{2+} and transfer it into a 10 cm petri dish using a bent tweezer.

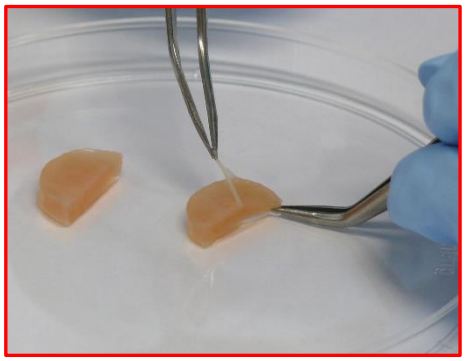
   While holding the skin model with the tweezer (see picture), cut the model with a scalpel into two halves.
   Optional: Store half of the model appropriately for further purposes, e.g. histology or RNA preparation.





2. Distribute evenly 300 µl Thermolysin solution (0.5 mg/ml) in the cavity of a 12-well plate. Transfer both tissue parts carefully “on top” of the enzyme solution, making sure that no Thermolysin gets on top of the tissue. Incubate for 2h at 4 °C.

3. Transfer the skin model into a 10 cm petri dish and fix the dermis of the tissue with tweezers. Carefully peel off the epidermis with a second pair of tweezers (see picture).



4. Transfer compartments into separate vessels for further processing, e.g. 3D Skin Comet assay, RNA or protein preparation (see separate protocols).