



## EZSep™ Melanocyte Isolation Kit

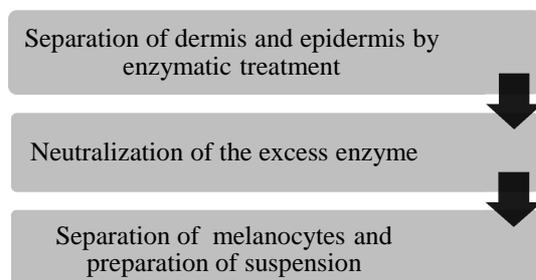
**Product Code: CCK001**

### 1. Introduction:

Melanocytes are cells that produce melanin and are located in the bottom layer of epidermis of skin, middle layer of the eye, the inner ear, meninges, bones and heart. Apart from being the major determinant of skin colour, melanin absorbs UV light and inhibits radical ion generation, protecting the skin from sun damage and aging. With the ever increasing risks of melanoma, skin depigmentation and several other diseases related to deficiency of melanin, intense research is focused on development of different treatment strategies to increase melanin production. One important approach in this research is the use of *in vitro* cultured melanocytes that are isolated from primary tissues and can be used for melanin production or wound/ sear healing. Various methods have been developed for isolation and culturing of melanocytes. EZSep™ Melanocyte Isolation Kit is based on the classical method of enzymatic dissociation of skin grafts at the epidermal-dermal interface and isolation of cells from dissociated tissue.

### 2. About the kit:

EZSep™ Melanocyte Isolation Kit is designed for easy isolation of melanocytes from skin grafts and helps to obtain a very high yield of cells. The procedure can be divided into three steps:



The dissociation reagent catalyses separation of dermal and epidermal layers in the graft tissue by hydrolysis of cellular attachment matrices. The provided neutralizing reagent binds to and inactivates the dissociation reagent as prolonged exposure to dissociation reagent may harm viability of isolated melanocytes. Melanocytes can then be suspended in the melanocyte-suspension-medium that

is enriched with the growth factors, amino acids, vitamins and other ingredients vital for maintaining the viability of melanocytes, *in vitro*.

### 3. Kit Contents:

This kit provides sufficient reagents for isolation of melanocytes from skin graft of dimensions 2cm x 4cm (typically specimens from skin biopsies)

Contents		Quantity	Storage
Code	Description		
CCK001(A)	Dissociation Mix	1 vial	2 - 8°C
CCK001(B)	Reconstitution buffer	1 x 13ml	2 - 8°C
CCK001(C)	Neutralizing reagent	1 x 3ml	2 - 8°C
CCK001(D)	Melanocyte suspension medium	1 x 25ml	2 - 8°C
CCK001(E)	Petri plates	2 Nos.	RT*
CCK001(F)	Conical bottom tubes	1 No	RT*
CCK001(G)	Syringe driven filter	1 No	RT*

\* RT- Room temperature

### 4. Materials required but not provided in the kit:

- Stainless steel pointed forceps (LA710)
- Stainless steel blunt forceps (LA707)
- Serological Pipettes - 5ml (PW1193)  
10 ml (PW1194)
- Pipette aid (Steripette) (LA692)
- Scissors
- Skin graft

### 5. Directions for use:

**Users are advised to review entire procedure before starting the assay**

Entire procedure must be carried out under sterile conditions inside a laminar flow hood or a biosafety cabinet.

#### 5.1. Preparation of dissociation reagent:

Dissolve the contents of dissociation mix in entire quantity of reconstitution buffer and filter sterilize using 0.22µ syringe filter CCK001(G). The dissociation mix will dissolve slowly in reconstitution

buffer. It is not advisable to use procedures such as vortexing or rigorous pipetting to make the solubilization faster.

## 5.2 Procedure for isolation of melanocytes from skin graft:

### Step I: Separation of dermis and epidermis by enzymatic treatment:

#### 1. Washing of the graft:

- a) Transfer the skin graft in a sterile conical bottom centrifuge tube. Add 4ml Dissociation reagent and 1ml Melanocyte suspension medium, one after the other.
- b) Mix the contents by shaking the tube several times and discard the solution.

#### 2. Dissociation:

- a) To the washed skin graft, add 6ml of Dissociation reagent and incubate the tube at in 5% CO<sub>2</sub> atmosphere at 37°C for 30 minutes. Swirl the tube intermittently (once every 10 minutes).
- b) After the incubation period is over, remove the solution with the help of sterile pipette.

### Step II: Neutralization

Add 3ml neutralizing reagent

### Step III: Separation of melanocytes and preparation of suspension:

#### 1. Separation:

- a) Transfer the treated graft in a sterile Petri plate. A small volume of the dissociation reagent-neutralizing reagent mixture is carried forward and helps in the subsequent steps.
- b) Hold the skin graft at one corner with blunt forceps by gently pressing it onto the Petri plate. (*Note: Excess pressure can tear and damage the cells, hence handle the graft gently.*)
- c) Hold the pointed forceps in other hand and gently scrape the skin surface to separate epidermis from dermis layer.
- d) Keep the epidermis in a Petri plate and transfer the dermal portion to the centrifuge tube.
- e) Scrape the ventral side of the epidermis kept in Petri plate with the help of pointed forceps into small pieces. Mince these pieces in smaller fragments of about 1-2mm size.

- f) Vortex the centrifuge tube containing dermal portion for 30 seconds.

*Note: Vortexing facilitates separation of epidermal cells that are closely attached to the dermal cells.*

- g) Remove the dermal pieces with the help of Pasteur pipette and discard them.
- h) Transfer the solution containing minced epidermal fragments from Petri dish to the centrifuge tube already containing the separated cells.
- i) Vortex the tube for 30 seconds. This will free the melanocytes that are still attached to epidermis. Discard the remaining epidermal fragments.
- j) Centrifuge the solution at 1500rpm for 5 minutes to get the pellet of melanocytes.

#### 2. Preparation of melanocyte suspension:

- a) Suspend the pellet in 2ml Melanocyte suspension medium and shake gently.
- b) Centrifuge the resuspended melanocytes at 1500rpm for 5 minutes.
- c) Discard the supernatant and repeat the step (j).
- d) Isolated melanocytes can now be used for further applications.

*(Note: In our hands, this procedure usually results in co-purification of a variable percentage of fibroblasts and keratinocytes.)*

## 6. Storage and Shelf life:

- Upon receipt, store the reagents at 2-8°C and plastic ware at room temperature.
- Store the reconstituted dissociation reagent at -20°C.
- Use before expiry date given on the label.

## 7. Important Notes:

- Efficiency of isolation depends on the source and quality of the skin graft as well as technical skills of the personnel.
- Coarse handling can lead to tissue damage and ultimately the cell death. Hence handling should be very supple.

## 8. Available pack sizes of EZSep™ Melanocyte Isolation Kit:

Code	Pack size
CCK001-1PR	1 Preparation
CCK001-5 x 1PR	5 x 1 Preparation

### Disclaimer:

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