Version 1 Last updated 31 October 2018

ab241012 Basement Membrane Extract Kit for 3D Cell Culture

For spheroid formation assays in adherent and suspension cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Basement Membrane Extract Kit for 3D Cell Culture (ab241012) offers 3D cell culture matrices to meet the needs and requirements of various research fields. This kits provide a standardized, yet user friendly and adaptable to high-throughput strategy for setting up spheroid formations, 3D cell cultures and pharmacological studies.

2. Protocol Summary

Prepare reagents and grow cells in appropriate culture conditions.

Harvest, centrifuge and count the number of cells. Resuspend cells in media at the concentration of 2 x 10^6 cells/ml.

Add 500 µL of cells to 4.5 mL of BME Matrix. Mix and add 50 µL of cell mixture.

To solidify the matrix, incubate the entire plate in 37°C with cell and matrix mixture for 30 min.

Add 200-250 µL of media and allow cells to grow and form spheroids in 37°C incubator. Change media every 2-3 days.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
BME Matrix	5 mL	-20°C
Wash Buffer	100 mL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Cell culture media
- 96-well plate (sterile, clear-bottom)
- Microscope
- Matrix Dissociation Buffer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 BME Matrix:

Ready to use as supplied. Aliquot and store at -20°C. Avoid multiple freeze/thaw. Use within two months. Thaw and keep on ice before use.

6.2 Wash Buffer:

Read to use as supplied. Store at -20°C or 4°C. Stable for six months after the first thaw. Bring to room temperature (RT) before use.

7. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

7.1 Cells:

- Grow cells in appropriate media and culture conditions. Adherent cells should be cultured to ~80% confluency.
- Harvest cells and centrifuge at 1,000 x *g*, for 5 min. Resuspend the cell pellet in Wash Buffer and count the number of cells using a hemocytometer or an automated cell counter.
- Resuspend cells in 500 µL of media at the concentration of 2 x 10^6 cells/ml, and keep cells on ice.

7.2 Matrix Preparation:

- Thaw matrix, and perform the assay on ice at all time.
- For a 96 well-plate, add 500 µL of resuspended cells from step 7.1 to 4.5 mL of thawed BME Matrix.
- Mix gently by pipetting, and add 50 µL of cell mixture to each well to get 10,000 cells per well.
- To solidify the matrix, incubate the entire plate in 37°C with cell and matrix mixture for 30 minutes.
- After the incubation time, add 200-250 µL of appropriate media and allow cells to grow and form spheroids in 37°C incubator for a set amount of days depending on experimental set up.
- Change media every 2-3 days.

ΔNote:

a. For a scaled-down experiment to 10 wells, add 55 µL of cells in media (2 x 10^6 cells/mL) to 495 µL of BME Matrix. Next, add 50 µL of cell mixture to each well to get 10,000 cells per well.
b. Cells typically form spheroids in matrix after 1 week.
c. BME Matrix remains stable for up to 3 weeks in culture. Vacuum removal of buffer or media could aspirate some or the entire matrix and can cause loss of samples. To avoid rupture of matrix, removal of buffer and media, by carefully pipetting from side of well, is strongly recommended.

7.3 Matrix Dissociation (optional):

- Matrix Dissociation Buffer is not provided. Add 200-250 µL of Matrix Dissociation Solution. Incubate at RT for 5-10 minutes and then pipet up and down with 1 mL tip until matrix is dissolved. Move the cells and solution to 1.5 mL Eppendorf tubes. To neutralize the Matrix Dissociation Solution, add 1 mL of Wash Buffer to each tube and centrifuge at 1,000 x *g*, for 5 minutes. Resuspend cells in media for use in assay of interest.

8. Typical Data

Typical data provided **for demonstration purposes only**.

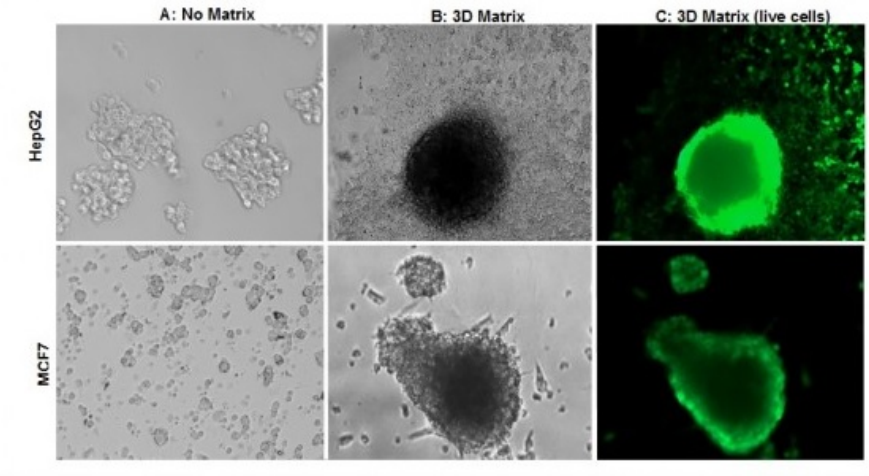

Figure 1. HepG2 and MCF7 cells in No Matrix (A) and 3D BME Matrix (B). Cells were cultured in BME Matrix for 21 days, and successfully formed spheroids. Media was changed every 2-3 days as per protocol. The Calcein AM staining (C) indicates that cell viability is not affected while culturing in matrix for a long period of time. *Note: Calcein AM is not included in the kit.*

9. Notes

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