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## CHANGE HISTORY

| Revision | Description |
|---|---|
| | Internal SOP used by PDMR In Vitro Laboratory |
| 10/15/2017 | Standardize SOP for posting to PDMR SharePoint site for use by designated NCI intramural laboratories |
| 5/14/2018 | Updated reference SOPs and Purpose/Scope section |
| 9/6/2018 | Clarify steps in Matrigel coating and length of time for storage before use. |
| 1/16/2019 | Added the need for Pen/Strep in the coating solution. Streamlined protocol for readability. |
| 2/28/2022 | Added protocol for coating flasks with Basement Membrane Extract (BME) |

## RELATED SOPS

| |
|---|
| SOP30103: Initial Culture, Sub-culture, and Cryopreservation of Adherent Patient-Derived Tumor Cultures (PDCs) |
| SOP30105: Initial Culture and Sub-culture of Patient-Derived Cancer-Associated Fibroblasts (CAFs) |

## 1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes preparation of Matrigel-coated or BME-coated plates for successful thawing and early culture of adherent Patient-Derived Tumor Cultures (PDCs) and Cancer-Associated Fibroblasts (CAFs) under BSL-2 safety criteria. This SOP is used/performed by the Biological Testing Branch (BTB) at NCI-Frederick, Frederick National Laboratory for Cancer Research.

## 2.0 SAFETY

BTB treats all patient-derived in vitro cell cultures under Biosafety Level 2 (BSL2) conditions even when PCR-based screening has not detected the presence of a known set of human pathogens. All work is conducted in a biological safety cabinet (BSC) using personal protective equipment and avoiding the use of sharps where possible. All materials potentially exposed to the cell cultures are disinfected by exposure to a 10% bleach solution for a minimum of 10 minutes, double bagging for autoclaving or incineration. Consult with your facility safety professionals regarding the safe handling of BSL2 studies.

## 3.0 CLEAN-UP

**3.1** All materials coming into contact with patient tissue as well as the mice carrying patient tumor samples are treated as a potential health threat (BSL-2 precautions) since the human tissues could retain human pathogenic agents even if they do not replicate in mouse cells (e.g., EBV, HPV, etc).

**3.2** Flush/soak any items (e.g., tubes, syringes, petri dishes, lab mats, etc) that were in contact with human tissue with disinfectant (e.g., 10% bleach, commercial hydrogen peroxide disinfectant, 2% Virkon®) for a minimum of 10 minutes before disposal in biohazard waste or sharps containers (follow institutional guidelines and manufacturer's recommendations).

**3.3** For items that can't be rinsed (e.g., micropipettors), wipe down thoroughly with bleach-soaked gauze or other appropriate disinfectants.

## 4.0 EQUIPMENT

**4.1** Equipment

**4.1.1** 50-mL, 25-mL, 10-mL, 5-mL pipettes, sterile

**4.1.2** 15 and 50-mL polypropylene tubes, sterile

**4.1.3** Tissue Culture flasks, sterile, vented

**4.1.4** Pipetman and sterile tips

**4.1.5** Waste container Bleach (Clorox, 5.25% Hypochlorite) diluted 1:10, 2% Virkon®, or similar disinfectant

**4.1.6** Refrigerator (4°C) and freezer (–20°C)

**4.1.7** 37°C Incubator (5% $CO_2$, humidified)

**4.1.8** Biological Safety Cabinet (BSC) meeting biosafety level 2 (BSL2) standards

**4.1.9** Personal Protective Equipment (PPE) at a minimum laboratory coat, with fitted sleeves, latex or nitrile gloves and safety glasses

## 5.0 PROTOCOL 1: MATRIGEL®-COATED FLASKS

**5.1** Reagents

**5.1.1** 1X HAM's F-12 Nutrient Mix, with L-glutamine (Invitrogen, Cat#: 11765-047) with 100 U/mL Pen/Strep final (Invitrogen, Cat#: 1514022, 10000 U/mL)

**5.1.2** Matrigel® Matrix

5.1.2.1 Matrigel®, High concentration (BD Biosciences, Cat#: 354248)

**OR**

5.1.2.2 Matrigel®, Standard concentration (BD Biosciences, Cat# 354234)

**IMPORTANT**: All Matrigel® purchases should be submitted specifying PCR-tested LDEV-Negative Matrigel®. If not, there is a possibility of LDEV contamination which can result in LDEV+ tumors

**5.2** Prepare Matrigel® Working Solution

**5.2.1** Chill pipettes and conical tubes in a -70°C freezer overnight and then place on wet ice prior to use.

**5.2.2** Thaw Matrigel® overnight by placing a vial of Matrigel, buried in ice, in the refrigerator.

**5.2.3** Using cold pipettes and tubes, make a Matrigel® Working Solution with 1X HAM's F-12 Nutrient Mix, with L-glutamine, + Pen/Strep.

5.2.3.1 Make a 25% Matrigel Working Solution if using High Concentration

5.2.3.2 Make a 50% Matrigel Working Solution if using Standard Concentration

**5.2.4** Aliquot Matrigel® Working Solution either as 5-mL aliquots into chilled sterile 15-mL conical tubes or 1-mL aliquots into 2-mL sterile screw-capped tubes and place into a -20°C freezer.

**5.3** Preparation of Matrigel®-coated flasks

**5.3.1** The day before coating, remove the appropriate number of Matrigel® Working Solution aliquots (prepared in Section 5.2) from the freezer and thaw overnight buried in ice, in the refrigerator.

**5.3.2** The recommended volume of 2.5% Matrigel needed per well/flask are:

| **Plate/Flask site** | **Volume 2.5% Matrigel®/well or flask** |
|---|---|
| 96-well plate | 75 µL/well |
| 24-well plate | 0.3 mL/well |
| 6-well plate | 2 mL/well |
| T25 flask | 3.0 mL |
| T75 flask | 6.0 mL |
| T162 flask | 8.0 mL |

**5.3.3** Prepare a 2.5% Matrigel solution using the 25% or 50% Matrigel Working Solution in 1X HAM's F-12 Nutrient Mix, with L-glutamine, + Pen/Strep. Keep solution chilled.

**5.3.4** Coat Tissue Culture Plates/Flasks with 2.5% Matrigel® Solution

5.3.4.1 Matrigel® coated plates/flasks should be prepared at least 1 hour before use.

5.3.4.2 Using sterile procedures, coat the growth surface of the plate/flask using the recommended volume based on the size of the well/flask (Section 5.3.2). Be sure to rock the plate back and forth to completely coat the surface of the plate/flask.

5.3.4.3 Incubate plates/flasks for a minimum of 30 minutes at ambient temperature for polymerization. This process can be enhanced by incubating in a 37°C incubator.

5.3.4.4 Immediately before use, remove excess media from the flask/plate and discard taking care to not dislodge the Matrigel® coating.

- Flasks can be prepared up to 7 days before use and stored at 4°C following the polymerization step (leave excess media on flask/plate until use). Bring to ambient temperature or 37°C before adding cells.

## 6.0 PROTOCOL 2: BME-COATED FLASKS

**6.1** Reagents

**6.1.1** 1X HAM's F-12 Nutrient Mix, with L-glutamine (Invitrogen, Cat#: 11765-047) with 100 U/mL Pen/Strep final (Invitrogen, Cat#: 1514022, 10000 U/mL)

**6.1.2** Cultrex Basement Membrane Extract (BME), PathClear®, with Phenol Red, 8-12 mg/mL stock (R&D Systems, Cat# 3432-005-01P)

6.1.2.1 A lot-specific Certificate of Analysis is included in each Cultrex BME shipment noting exact protein concentration.

6.1.2.2 Phenol Red is used as a visual indicator of coated plates

**6.2** Prepare Cultrex BME Working Solution

**6.2.1** Thaw Cultrex BME overnight by placing a vial of BME, buried in ice, in the refrigerator.

**6.2.2** Using pipettes and chilled tubes, dilute the 100X Phenol Red solution to 1X in BME. Note: pipettes can be room temperature

**6.2.3** Aliquot the Cultrex BME solution with 1X Phenol Red either as 5-mL aliquots into chilled sterile 15-mL conical tubes or 1-mL aliquots into 2-mL sterile screw-capped tubes and place into a -70°C freezer.

**6.3** Preparation of Cultrex BME-coated Flasks

**6.3.1** The day before coating, remove the appropriate number of Cultrex BME solution with 1X Phenol Red aliquots (prepared in Section 6.2) from the freezer and thaw overnight buried in ice, in the refrigerator.

**6.3.2** The recommended volume of 2.5% BME needed per well/flask are:

| Plate/Flask site | Volume 2.5% BME/well or flask |
|---|---|
| 96-well plate | 75 µL/well |
| 24-well plate | 0.3 mL/well |
| 6-well plate | 2 mL/well |
| T25 flask | 3.0 mL |
| T75 flask | 6.0 mL |
| T162 flask | 8.0 mL |

**6.3.3** Prepare a 0.46-0.48 mg/mL BME Working Solution containing ice-cold 1X Phenol Red with 1X HAM's F-12 Nutrient Mix, with L-glutamine, + Pen/Strep. Keep solution chilled.

**6.3.4** Coat Tissue Culture Plates/Flasks with 0.46-0.48 mg/mL BME Working Solution

6.3.4.1 BME coated plates/flasks should be prepared at least 1 hour before use.

6.3.4.2 Using sterile procedures, coat the growth surface of the plate/flask using the recommended volume based on the size of the well/flask (Section 6.3.2). Be sure to rock the plate back and forth to completely coat the surface of the plate/flask.

6.3.4.3 Incubate plates/flasks for a minimum of 1 hour at ambient temperature for polymerization. This process can be enhanced by incubating in a 37°C incubator.

6.3.4.4 Immediately before use, remove excess media from the flask/plate and discard.

- Flasks can be prepared up to 7 days before use and stored at 4°C following the polymerization step (leave excess media on flask/plate until use). Bring to ambient or 37°C before adding cells.