Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.800.492.1110 (US) or +1.978.442.2200.
ScientificSupport@corning.com, www.corning.com/lifesciences

CORNING

GUIDELINES FOR USE

PRODUCT Corning® Matrigel® matrix- 3D plate

FORMATS 384 well black/clear (356256); 384 well white/clear (356257, 356258); 96 well black/clear (356259).

BACKGROUND Implementation of 3D cell culture in drug discovery has been increasing steadily as it lends itself to a more physiological model. Therefore, anti-cancer drug screening using spheroids, tumoroids and organoids are considered better predictors of *in vivo* drug responses[1-3].

The Corning Matrigel matrix- 3D plate provides an *in vitro* assay format that allows for growth of cells in 3D for drug discovery applications. These high-throughput plates contain unpolymerized phenol-red-free Matrigel in each well and can be used with 'sandwich' or 'embedded' workflows to generate 3D cultures[4-5]. Corning Matrigel matrix is a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) tumor that gels at room temperature to form a genuine reconstituted basement membrane[6]. The major components of Corning Matrigel matrix are laminin, collagen IV, entactin, and heparan sulfate proteoglycan, growth factors, collagenases, plasminogen activators, and other undefined components[7-9].

Corning Matrigel Matrix-3D plates provide a consistent and easier workflow solution for forming 3D, polarized structures without the need to self-coat.

STORAGE Materials should be stored at -20°C in the original packaging. DO NOT STORE IN FROST-FREE OR -70°C FREEZER.

EXPIRATION See Certificate of Analysis

THAWING Remove box from -20°C storage and allow the unopened foil package containing plate to thaw at 4°C overnight on a flat surface. Do not stack plates during thawing.

CELL SEEDING & GELATION **Protocol** choice shall be empirically determined based on cell type, culture conditions and application.

Sandwich protocol (general):
For sandwich/overlay methods, cells are typically grown on a polymerized Matrigel layer and result in 3D cultures that are in a narrower focal plane making it ideal for imaging applications.

- After removing thawed plate from package, allow Matrigel Matrix in plate to polymerize for 30 – 60 minutes at 37°C / 5% CO_2 until the cells are ready to be seeded.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for use in humans.
For a listing of trademarks, visit www.corning.com/clstrademarks.
All other trademarks in this document are the property of their respective owners.
© 2019 Corning Incorporated

- While plates are polymerizing, prepare cell suspension for seeding.
    - Depending on the application and/or cell type, a dilute concentration of Matrigel (titrate between 0- 0.45 mg/mL in final volume/well) may be needed in the cell suspension.
- Seed cells onto polymerized Matrigel by carefully adding 50-200 µl of cell suspension per 96 well and 10-60 µl of cell suspension per 384 well. Take care not to disturb polymerized Matrigel.
- Incubate plate at 37°C in a humidified CO_2 incubator.
- Culture cells for desired period, refreshing medium as needed for your 3D application.

Embedded protocol (general)
For embedded cultures, cells may be added on top OR gently mixed with the un-polymerized Matrigel contained in each well.

- After thawing, hold plates at 4°C / on ice until ready to seed with cells.
- Then, prepare a dense cell suspension and hold at 4°C / on ice until ready to add to plate
  for 96 well plate suggested volume is 5-20 µl/well
  for 384 well plate suggested volume is 2-5 µl/well
- Add the cell suspension to thawed plates at 4°C/ on ice by:
    - Carefully dispensing on top of thawed Matrigel layer.
        - TIP: *Ensure plates are kept cold during cell seeding to prevent premature gelation.*
        - *DO add cell suspension to center of each well and do NOT apply a swirling mixing motion as cells can seep through sides of the well causing growth of cells in 2D.*
        - *Holding seeded plates at 4°C in a refrigerator for an additional 30 min before polymerizing can improve embedding results.*

  OR
    - Gently mixing cells with thawed Matrigel in each well
        - TIP*: When cells are mixed using pipette, care must be taken to not introduce bubbles into the cell-Matrigel mixture*
- Then, polymerize plates containing cell suspensions for 30 – 60 minutes at 37°C / 5% CO_2, add growth medium and culture as required.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for use in humans.
For a listing of trademarks, visit www.corning.com/clstrademarks.
All other trademarks in this document are the property of their respective owners.
© 2019 Corning Incorporated

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices.

REFERENCES:

1. Fang Y. et. al. Three-Dimensional Cell Cultures in Drug Discovery and Development. SLAS Discov. 22(5):456 (2017)
2. Liu H-D et.al. Organoid of ovarian cancer: genomic analysis and drug screening. Clin. Transl. Oncol. (2020) https://doi.org/10.1007/s12094-019-02276-8
3. Weeber F. et.al. Tumor Organoids as a Pre-clinical Cancer Model for Drug Discovery. Cell Chem. Biol. 24:1092 (2017).
4. Li L et.al. Optimizing a 3D Culture System to Study the Interaction between Epithelial Breast Cancer and Its Surrounding Fibroblasts. J. Cancer 2: 458-466 (2011)
5. Corning® Matrigel® Matrix-3D plates for High-throughput 3D Assays. CLS-AN-572
6. Kleinman HK, et al. Basement membrane complexes with biological activity. Biochemistry, 25:312 (1986).
7. Kleinman HK, et al. Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. Biochemistry, 21:6188 (1982).
8. Bissell DM, et al. Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. J Clin Invest, 79(3):801 (1987).
9. Vukicevic S, et al. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res, 202:1 (1992).

California Proposition 65 Notice

WARNING:

This product contains a chemical known to the state of California to cause cancer. Component: Chloroform.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for use in humans.

For a listing of trademarks, visit www.corning.com/clstrademarks.
All other trademarks in this document are the property of their respective owners.
© 2019 Corning Incorporated