BD™ PuraMatrix™ Peptide Hydrogel

Catalog No. 354250

Guidelines for Use

FOR RESEARCH USE ONLY.
NOT FOR CLINICAL, DIAGNOSTIC. OR THERAPEUTIC PROCEDURES.
NOT FOR USE IN HUMANS.

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INTENDED USE

BD™ PuraMatrix™ Peptide Hydrogel (BD™ PuraMatrix™) is a synthetic matrix that is used to create defined three dimensional (3D) microenvironments for a variety of cell culture experiments. To achieve optimal cell growth and differentiation, it is necessary to determine the appropriate mixture of this material and bioactive molecules (e.g., growth factors, extracellular matrix (ECM) proteins, and/or other molecules). BD PuraMatrix consists of standard amino acids (1% w/v) and 99% water. Under physiological conditions, the peptide component of BD PuraMatrix self-assembles into a 3D hydrogel that exhibits a nanometer scale fibrous structure with an average pore size of 50-200 nm. The hydrogel is readily formed in a culture dish, multiwell plate, or cell culture insert.

BD PuraMatrix provides the benefits of 3D cell culture while allowing more straightforward 2D plating. Many cell types form appropriate 3D morphologies when plated on the surface of BD PuraMatrix, which simplifies the work required to develop 3D *in vitro* model systems compared with cell encapsulation or sandwich culture systems. Research has shown that cells forming 3D structures often resemble their *in vivo* counterparts more closely when compared to cells grown in 2D. BD PuraMatrix allows defined microenvironments to better recapitulate the *in vivo* milieu, which should improve the predictive quality of results obtained from assays conducted in a high-throughput context.

The hydrogel has been shown to support the differentiation of hepatocyte progenitor cells[1], rat pheochromocytoma cells (PC12)[2] and hippocampal neurons[3], as well as enabling endothelial cell tubulogenesis[4]. Studies have also demonstrated that BD PuraMatrix supports the attachment of a variety of primary (e.g., neuronal, fibroblast, and keratinocyte) and transformed (e.g., MG-63, SH-SY5Y, HEK293, and NIH3T3) cell types[5,6]. Other potential applications include stem cell proliferation and differentiation, tumor cell migration, and *in vivo* analyses of tissue regeneration. BD PuraMatrix is biocompatible, resorbable, and devoid of animal-derived material and pathogens. For *in vivo* studies in animals, the soluble material can be injected and will subsequently form a 3D hydrogel upon contact with the physiological environment.

References

1. Semino, C.E., et al., *Differentiation* **71**:262 (2003).
2. Holmes, T.C., et al., *PNAS USA* **97**:6728 (2000).
3. Semino, C.E., et al., *Tissue Engineering* **10**:643 (2004).
4. Narmoneva, D., et al., *Biomaterials* **26**:4837 (2005).
5. Zhang, S., et al., *Biomaterials* **16**:1385 (1995).
6. Thonhoff, J.R., et al., *Brain Research* **1187**:42 (2008).

For additional information, visit our website at:
bdbiosciences.com/PuraMatrix

MATERIALS PROVIDED

- BD™ PuraMatrix™ Peptide Hydrogel (BD Cat. No. 354250) packaged in one vial containing 1% solution (w/v) of purified synthetic peptide (5 ml per vial)

SUPPLEMENTAL/OPTIONAL MATERIALS (NOT SUPPLIED)

- Tissue culture media for cells of interest
- 20% sucrose in distilled water (dH_2O), sterile filtered (prepare 10% sucrose stock by diluting with sterile water)
- ECMs, growth factors, and cytokines
- BD Falcon™ Cell Culture Inserts and BD Falcon™ Cell Culture Plates
- For information about available products from BD Biosciences, visit our website at: bdbiosciences.com

Hints For Optimal Results with BD PuraMatrix Peptide Hydrogel

PRECAUTIONS

- **GELATION:** Since the gelation of this material is initiated by salt concentrations ≥1 mM, this process may be promoted by salt-containing buffers and/or media. Therefore, do not combine BD PuraMatrix with salt-containing buffers and/or media until gelation is desired. Once the gel is formed, any subsequent mixing of the sample will disrupt the structure of the hydrogel.

- **VISCOSITY:** Decrease the viscosity of the stock solution (1% w/v) by vortexing or sonication for 30 minutes in a bath sonicator every time the stock is used. If air bubbles are present, centrifuge an aliquot of the stock in a 1.5 ml microtube at full speed.

- **pH and OSMOLARITY:** Since BD PuraMatrix exhibits a pH of 2-2.5, work quickly to minimize the amount of time that cells are in contact with the material prior to the addition of media when performing cell encapsulation experiments. Addition of isoosmotic sucrose to the cell suspension as well as to the BD PuraMatrix, will help protect the cells until the pH is normalized by equilibration with tissue culture media. For details, see **Parts D and E (Cell Encapsulation Protocols)**.

- **HANDLING:**
  - Since BD PuraMatrix forms a relatively soft fibrous matrix, it is necessary to handle the material very carefully when performing media changes. **Do not use vacuum aspiration** to remove media from above the hydrogel. Avoid direct contact with the hydrogel.

  - When adding media and/or other components to the sample, place the end of the pipette tip towards the top of the culture vessel wall.

  - When using culture vessels with large surface areas (e.g., ≥35 mm dishes), perform fluid handling steps with extreme care to avoid disruption of the hydrogel.

GENERAL USE

- See table on page 15 to determine appropriate volume for desired BD Falcon™ Cell Culture Insert or Multiwell Plate format.

- BD™ PuraMatrix™ can be used in the undiluted form at a concentration of 1% (w/v) or diluted as described in **PROCEDURES FOR USE**. Concentrations below 0.5% are suitable for many applications. For example, we recommend 0.25% for surface plating of most cell types, and a final concentration of 0.15% for encapsulating neurons and endothelial cells.

- To supplement the hydrogel with ECMs, growth factors, and/or other bioactive molecules, refer to **Part C (ECM Supplementation Protocols)**.

- If using serum-free media for culturing cells, it may be necessary to deactivate the trypsin following cell isolation by trypsinization.

IMPORTANT: To achieve optimal cell growth and differentiation, it is necessary to determine the appropriate mixture of BD PuraMatrix Peptide Hydrogel and bioactive molecules (e.g., growth factors, ECM proteins, and/or other molecules). In general, it may be preferable to add growth factors to the media rather than encapsulate them in the hydrogel for most applications.

PROCEDURES FOR USE

A) Surface Plating of Adherent Cells in Cell Culture Plates

Note: Many cell types that are plated on the surface of BD PuraMatrix can assume the appropriate and expected 3D structures (e.g., hepatoycyte spheroids, embryonic stem cell colonies and embryoid bodies, neuronal cell will branch). The 2D surface plating method is an ideal tool for screening both morphological and functional characteristics of any given cell type.

BD PuraMatrix Preparation and Gel Formation

1. Decrease the viscosity of the BD PuraMatrix stock solution (1% w/v) by vortexing or by sonication in a bath sonicator as described above. Prepare the appropriate volume of BD PuraMatrix in a microtube by diluting the stock with sterile dH_2O.

2. Prepare a sufficient volume of the desired concentration of BD PuraMatrix (e.g., 250 µl for a 24-well plate or 50 µl for a 96-well plate) by dilution with sterile dH_2O. Add to the surface of the well and promote gelation by carefully and slowly adding medium to each well (e.g., 500 µl for a 24-well plate or 100 µl for a 96-well plate).

3. Place plate in an incubator for 30-60 minutes to complete the gelation of the BD PuraMatrix. For higher percentage hydrogels, 30 minutes is sufficient; for 0.15-0.25% hydrogels, a 1-hour equilibration is preferable. After the hydrogel has assembled, carefully change the medium as described above. Change the medium two times over a period of 1 hour to equilibrate the growth environment to physiological pH. If necessary, the equilibrated sample can be stored overnight at 37ºC after the final media change.

Cell Plating

Trypsinize cells and spin down desired number (typically 4-16x10^4 cells/cm^2 final concentration for most cell types). Resuspend cells in tissue culture media at an appropriate concentration of cells in a final volume of 200-350 µl. Carefully add the cell suspension to the top of the hydrogel.

B) Surface Plating of Adherent Cells in Cell Culture Inserts

The following protocol is based on the use of a BD Falcon™ 24-well Cell Culture Insert (BD Cat. No.353095) and a BD Falcon™ Cell Culture Insert Companion Plate (BD Cat. No. 353504).

BD PuraMatrix Preparation and Gel Formation

1. Decrease the viscosity of the BD PuraMatrix stock solution (1% w/v) by vortexing or by sonication in a bath sonicator as described above. Prepare the appropriate volume of BD PuraMatrix in a microtube by diluting the stock with sterile dH_2O. We recommend a total volume of 100 µl per cell culture insert at a final concentration of 0.25% for most cell types. Mix by gentle pipetting.

2. Place desired number of cell culture inserts in the insert companion plate. Add 250 µl of media to the lower chambers of each 24-well insert by pipetting down the side of the outer well between the insert and the well. Pipet carefully to avoid bubbles under the insert. The media should just touch the bottom of the insert. **See Table on page 15 for cell culture insert volume recommendations.**

3. Pipet 100 µl of diluted BD PuraMatrix into the center of each of the inserts. Wait at least 30 minutes for the BD PuraMatrix to gel.

4. VERY CAREFULLY layer 400 µl media onto the gel with a pipet tip (use a P200 pipetter and add 200 µl (two times) for smaller droplet size) by running it slowly down into the inner wall of the insert.

5. Follow steps 10-13 in **Part E** to complete pH equilibration of the BD PuraMatrix prior to cell plating.

Cell Plating

1. Trypsinize cells and spin down desired number (typically 4-16x10^4 cells/cm^2 final concentration for most cell types). Resuspend cells in tissue culture media at an appropriate concentration of cells in a final volume of 200-350 µl.

2. Remove the media above the hydrogel and from the well as described above.

3. GENTLY pipet the cells into the inserts using a P200 pipetter, being careful not to disturb the hydrogel.

4. Add 700-900 µl of media below each insert.

5. For further cell feeding, change the media VERY GENTLY approximately every two days as described above.

C) ECM Preparation for Supplementation of BD™ PuraMatrix™ Peptide Hydrogel

Note: Prior to use, decrease the viscosity of the BD™ PuraMatrix™ stock solution (1% w/v) by vortexing or by sonication in a bath sonicator for 30 minutes every time the stock is used. If air bubbles are present, centrifuge an aliquot of the stock solution in a 1.5 ml microtube.

ECM Supplementation for Surface Plating of Cells

Fibronectin Protocol: (Since fibronectin is not soluble in the absence of salt, it must be "coated" onto the fibers).

1. Resuspend 5 mg BD™ Fibronectin (BD Cat. No. 356008) in 5 ml sterile dH_2O.

2. Dialyze overnight at Room Temperature (RT) using 10,000 MW cutoff Slide-A-Lyzer® (Pierce Cat. No. 66453), against dPBS without Ca^{2+} and Mg^{2+}.

3. Prior to addition of fibronectin, mix BD PuraMatrix thoroughly with gentle pipetting and add to well of appropriate plate at desired concentration. **See Table on page 15 for suggested volumes**.

4. Gel BD PuraMatrix by adding Fibronectin/PBS solution (1 mg/ml) on top of the BD PuraMatrix for 60 minutes.

5. Carefully rinse with cell culture media for 5 minutes, aspirate and then carefully add cells to the top of the matrix.

Collagen I Protocol: (Collagen I is soluble in 1 mM HCl and thus can be incorporated into the BD PuraMatrix before gelation):

1. Dialyze collagen I (stock solution in acetic acid, BD Cat. No. 354236) using 10,000 MW cutoff Slide-A-Lyzer® against 1 mM HCl overnight at $4^{o}C$.

2. Add the required amount of collagen I/HCl to BD PuraMatrix and mix with gentle pipetting.

3. Add to well.

4. Gel with cell culture media by adding media carefully to side wall of cell culture insert or well of plate.

5. Once gel has formed, remove media carefully with a pipet and change two more times over the next one hour to equilibrate the BD PuraMatrix to physiological pH prior to plating the cells on top of the matrix.

Laminin Protocol: (Laminin is provided in 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.4).

1. Low concentration laminin [Laminin, mouse (BD Cat. No. 354232) and Ultra-pure Laminin, mouse (BD Cat. No. 354239)].

    a. Mix with cell culture media at the desired concentration.

    b. Carefully add the mixture directly on top of BD™ PuraMatrix™ (prepared in the presence or absence of cells) in the cell culture insert or plate to promote gelation.

2. Laminin/Entactin, High Concentration (HC)**,** 10 mg/ml (BD Cat. No. 354259).

    Add required volume of HC Laminin directly to BD PuraMatrix. At dilutions of HC Laminin that result in a final concentration of ≤1 mM salt, gelation of BD PuraMatrix will not occur. To induce gelation after the addition of Laminin, add cell culture media and equilibrate the pH as described in the collagen I protocol.

ECM Supplementation of BD PuraMatrix for Cell Encapsulation

Detailed instructions for cell encapsulation in BD PuraMatrix can be found in Parts D and E. These should be followed, with the modifications detailed below, to introduce ECMs into the cell encapsulation mixture.

For BD PuraMatrix concentrations ≤0.25%, a cell culture insert is recommended to facilitate media changes and ensure that the hydrogel is not disrupted. BD PuraMatrix concentrations below 0.25% are recommended for the encapsulation of neurons and endothelial cells. With other cell types, testing a range of hydrogel concentrations for encapsulation is recommended.

To add ECMs to cell encapsulation mixture for cell culture plates or cell culture inserts:

1. Prepare ECMs as described above.

2. Add laminin or collagen (fibronectin is not suitable for encapsulation, but it can be coated onto samples of cells that are encapsulated within BD PuraMatrix) to the cell/sucrose mixture to a final concentration of 1-5 µg/ml (2X desired final concentration).

3. For cell encapsulation, follow protocols in **Part D** (cell culture plates) or **Part E** (cell culture inserts).

D) 3D Cell Encapsulation in Cell Culture Plates (with or without ECM protein supplementation)

1. Decrease the viscosity of the BD PuraMatrix stock solution (1% w/v) by vortexing or by sonication in a bath sonicator for 30 minutes every time the stock is used. If air bubbles are present, centrifuge an aliquot of the stock solution in a 1.5 ml microtube.

2. Prepare the appropriate volume of desired concentration of BD PuraMatrix in a microtube by diluting the stock with sterile 20% sucrose to generate a 2X concentration of BD PuraMatrix in 10% sucrose. **See Table on page 15 for cell culture plate volume recommendations.**

3. Trypsinize cells and spin down desired number (typically $5x10^5$-$1x10^6$ cells/ml final concentration for most cell types).

4. Remove media from cell pellet and resuspend cells to desired density in sterile 10% sucrose.

5. Collect cells again by centrifugation and resuspend the cells in 10% sucrose at 2X the final desired cell concentration.

6. Add equal volumes of 2X BD™ PuraMatrix™ and 2X cell/sucrose mixture, mix, and then add to the center of the well carefully, without introducing bubbles.

7. Initiate gelation of the BD PuraMatrix by gently running culture media down the side of the well on top of the hydrogel.

8. Repeat until cells have been plated in all wells.

9. VERY GENTLY change the media two times over the next one hour to further equilibrate the pH of the hydrogel. DO NOT USE a vacuum aspirator and only remove about 2/3 to 3/4 of the media to avoid disrupting the BD PuraMatrix.

10. Cells can be fed as necessary using the procedure described in step 9.

E) 3D Cell Encapsulation in Cell Culture Inserts (with or without ECM protein supplementation)

The following protocol is based on the use of a BD Falcon™ 24-well Cell Culture Insert (BD Cat. No. 353095) and a BD Falcon™ Cell Culture Insert Companion Plate (BD Cat. No. 353504).

For BD PuraMatrix concentrations $\leq$0.25%, a cell culture insert is recommended to facilitate media changes and ensure that the hydrogel is not disrupted. BD PuraMatrix concentrations below 0.25% are recommended for the encapsulation of neurons and endothelial cells. With other cell types, testing a range of hydrogel concentrations for encapsulation is recommended.

BD PuraMatrix Preparation

1. Decrease the viscosity of the BD PuraMatrix stock solution (1% w/v) by vortexing or by sonication in a bath sonicator for 30 minutes every time the stock is used. If air bubbles are present, centrifuge an aliquot of the stock solution in a 1.5 ml microtube.

2. Prepare the appropriate volume of the desired concentration of BD PuraMatrix in a microtube by diluting the stock with sterile 20% sucrose to generate a 2X concentration of BD PuraMatrix in 10% sucrose. We recommend a total volume of 100 µl per 24-well cell culture insert (i.e., 50 µl of 2X BD PuraMatrix in 10% sucrose and 50 µl of cells at 2X in 10% sucrose). **See Table on page 15 for cell culture insert volume recommendations.**

    a. For example, to encapsulate neurons and endothelial cells in a final concentration of 0.15% BD PuraMatrix, prepare a stock of 0.30% BD PuraMatrix in 10% sucrose (for 1 ml, combine 500 µl of 20% sucrose, 300 µl BD PuraMatrix, and 200 µl of sterile H_2O).

3. Mix by gentle pipetting.

4. We recommend setting up a separate tube to mix the cells with BD™ PuraMatrix™ for each cell culture insert or well. The desired volume of BD PuraMatrix can be distributed to all the tubes at once.

Cell Seeding – Since BD PuraMatrix exhibits low pH, work quickly to minimize the amount of time that cells are in contact with the material prior to the addition of media. When plating multiple wells, we recommend that each microtube is prepared individually, as opposed to in a group, to avoid long exposure of the cells to the BD PuraMatrix in the absence of media.

1. Trypsinize cells and spin down desired number (typically $5x10^5$-$1x10^6$ cells/ml final concentration for most cell types).

2. Place desired number of cell culture inserts in the insert companion plate.

3. Add 250 µl of media to the lower chambers of each insert by pipetting down the side of the outer well between the insert and the well. Pipet carefully to avoid bubbles under the insert. The media should just touch the bottom of the insert (**Figure 1**).

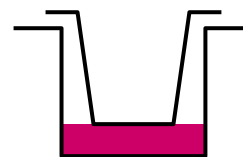


Figure 1

4. Remove media from cell pellet and resuspend cells to desired density in sterile 10% sucrose.

5. Collect cells again by centrifugation and resuspend the cells in 10% sucrose at 2X the final desired cell concentration.

6. QUICKLY mix diluted 2X BD PuraMatrix in 10% sucrose in a microtube with an equal volume of 2X cell suspension in 10% sucrose using gentle pipetting. Do not vortex or invert once cells are added.

7. QUICKLY pipet BD PuraMatrix/cell mixture into a 24-well insert (**Figure 2**). When dispensed into the center of the insert, the material will spread evenly over the surface.

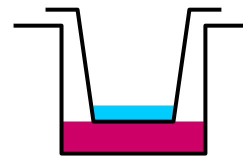


Figure 2

8. Wait five minutes for the BD PuraMatrix to settle in the well. During this period, set up the next insert as described in steps 6-7.

9. Return to the first insert and VERY CAREFULLY layer 400 µl media onto the gel with a pipet tip (use a P200 pipetter and add 200 µl (two times) for smaller droplet size) by running it slowly down the inner wall of the insert (**Figure 3**).

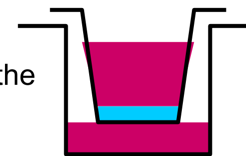
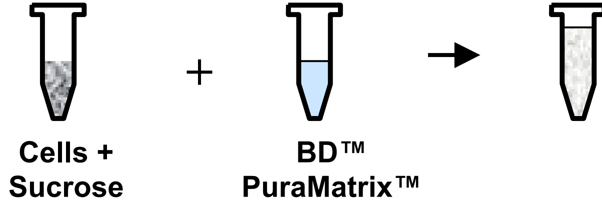
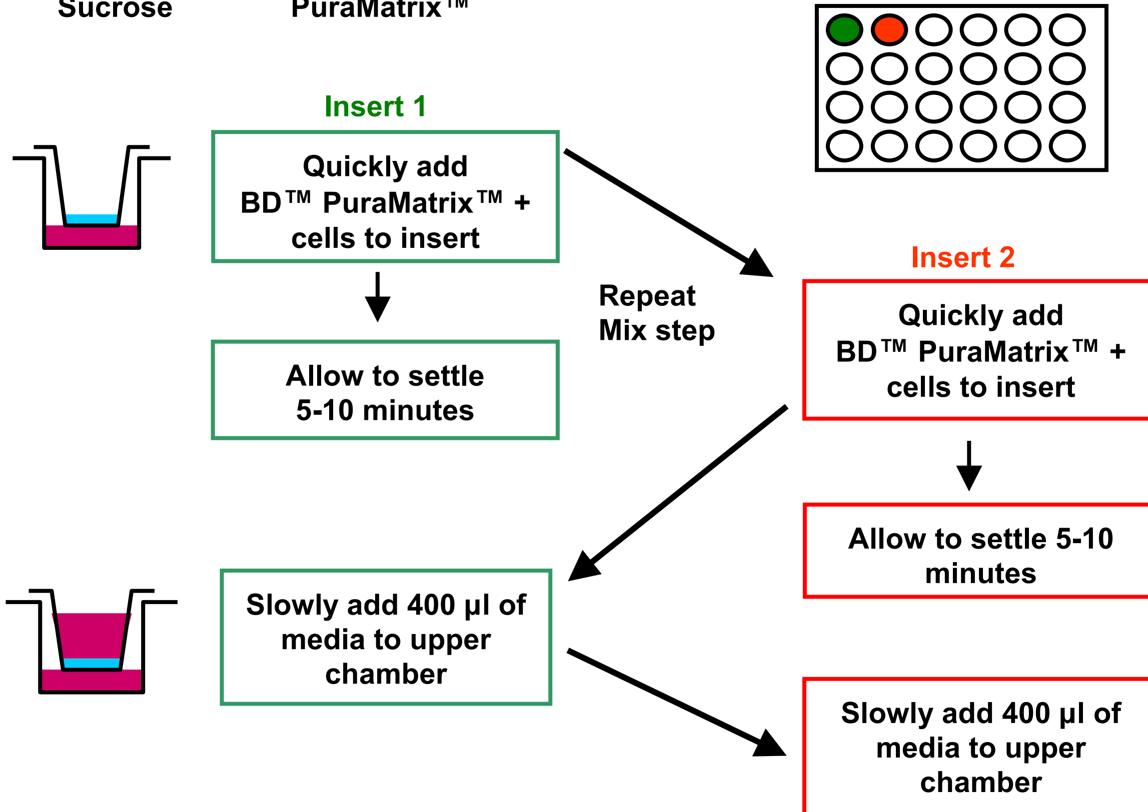


Figure 3

Flowchart of steps 6 through 9

Mix for each insert:





Repeat until all inserts are seeded, then continue with step 10 of the protocol.

10. When all inserts have been filled, place the plate in an incubator for 30-60 minutes to complete the gelation of the BD™ PuraMatrix™. For higher percentage hydrogels, 30 minutes is sufficient; for 0.15-0.25% hydrogels, a one-hour equilibration is preferable. The media levels may equilibrate to some extent between the insert and the well during this period (**Figure 4**).

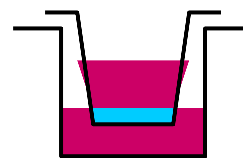


Figure 4

11. Change the media without disturbing the hydrogel. As the gelled BD PuraMatrix is difficult to see, this is best accomplished by removing approximately 2/3 of the media above the hydrogel with a P200 pipetter and keeping the tip above the bottom of the insert (**Figure 5**). DO NOT USE A VACUUM to aspirate media from above the hydrogel as it will disturb the BD PuraMatrix scaffold and cells.

    The media in the wells below the insert can be removed with a vacuum aspirator or a pipet tip.

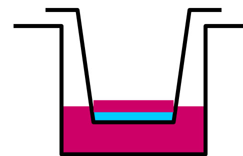


Figure 5

12. VERY GENTLY replace the media removed from the inserts, and also in the wells, as described above. Place the plate in the incubator for 30 minutes and then repeat the media change as described in step 11 and incubate again. This will equilibrate the pH of the BD PuraMatrix and remove excess sucrose. After the second change, place 200-350 µl of media into the insert and 700-900 µl into the well.

13. For further cell feeding, change the media VERY GENTLY approximately every two days as described above.

F) Cell Drop Culture Protocol

This technique, which allows the formation of small beads containing cells encapsulated within BD PuraMatrix, can be used as a quick screen for optimizing the conditions for encapsulating the cell type of interest within BD PuraMatrix (in the presence or absence of ECM proteins). The cell drop culture protocol can also be used to quickly assess cell viability, cell morphology, and/or immunostaining of cells encapsulated within BD PuraMatrix.

1. Decrease the viscosity of the BD PuraMatrix stock solution (1% w/v) by vortexing or by sonication in a bath sonicator for 30 minutes every time the stock is used. If air bubbles are present, centrifuge an aliquot of the stock solution in a 1.5 ml microtube.

2. Prepare the appropriate volume of BD PuraMatrix in a microtube by diluting the stock with sterile 20% sucrose to generate a solution with 2X the desired final concentration of BD PuraMatrix in 10% sucrose. Mix by gentle pipetting.

3. Grow cells to 75-80% confluence and harvest using standard procedures.

4. If ECM proteins are to be added to the drops, prepare as described in **Part C** (**ECM Supplementation Protocol)**. The final salt concentration should be ≤1 mM to prevent gelation of the BD™ PuraMatrix™.

5. Gently pellet the cells at 1000 x g, wash with 5 ml of 10% sucrose (filter sterilized, 0.2 µm membrane), re-pellet, and then resuspend with 10% sucrose to 2X desired final concentration (generally $5x10^5$ - $1x10^6$ cells/ml). Mix ECM proteins to 2X final concentration at this step. Use gentle pipetting to mix the sample.

6. Combine the cell/sucrose suspension with an equal volume of BD PuraMatrix and mix with gentle pipetting. Dispense 15 µl of the cell/hydrogel mixture along the side of the well on the surface of the media (1 ml of complete medium), which initiates the assembly of the hydrogel. Multiple "drops" can be dispensed in the well containing medium.

7. To assess cell viability after the desired time in culture, the drop culture can be analyzed using the LIVE/DEAD Viability/Cytotoxicity Assay Kit (Molecular Probes Cat. No. L-3224). Collect the cell/gel particles with a spatula from the surface of the media. Place the bead sample on a glass slide, and then add 50 µl of LIVE/DEAD Calcein AM and EthD-1 solution.

8. Incubate the slide for 30 minutes at room temperature and then examine using an imaging microscope for the presence of green (LIVE) and red (DEAD) cells.

G) Cell Recovery for Sub-Culturing or Biochemical Analyses

1. Mechanically disrupt BD PuraMatrix and cells in the well or cell culture insert by repeated pipetting of the media and gel.

2. Transfer to a 15 ml conical tube. Use another half to full volume of PBS to rinse out the well or cell culture insert and combine in the tube.

3. Centrifuge at low speed for five minutes. Discard supernatant. The pellet at the bottom of the tube contains cells and BD PuraMatrix fragments.

4. Resuspend pellet in 2 ml PBS, spin, and collect pellet again.

5. Resuspend pellet in 1 ml of trypsin-EDTA and incubate at 37ºC for 5-10 minutes. This will help separate cells that are still attached to each other.

6. Add 5 ml media and repellet.

7. Add 500 µl media (for replating) or PBS (for biochemical analyses) to the pellet and resuspend by pipetting. Centrifuge and discard supernatant. At this point, some cells may have residual "tags" of BD PuraMatrix attached which cannot be removed.

8. For biochemical and molecular analyses, add chosen buffer to pellet for cell solubilization.

9. For cell plating, transfer the pellet in media to a new cell culture plate or insert. Cells can be re-encapsulated (see **Parts D and E**), surface plated on a layer of BD™ PuraMatrix™ (see **Parts A and B**) or seeded directly onto a plastic plate (uncoated or coated with an ECM protein).

H) Fluorescent Staining of Samples Cultured with BD PuraMatrix Peptide Hydrogel

IMMUNOSTAINING

This protocol can be used with 3D or 2D surface cultures of cells in inserts or plates. For certain cell culture formats (e.g., 24- and 96-well microplates with a clear bottom), it may be possible to directly image the samples without transferring the gel to a glass slide. In some cases, it may be necessary to transfer the gel to a glass side for imaging analysis [e.g., when using a cell culture insert with translucent membrane (e.g., BD Cat. No. 353495)].

Note: To achieve a strong and specific signal, it is necessary to thoroughly block non-specific binding of antibodies to BD PuraMatrix as well as allowing diffusion of antibodies through the peptide scaffold. Therefore, extended blocking and incubation times, higher antibody concentrations, and multiple washes are required.

1. Gently remove media from culture well.

2. Fix cells in gels in 4% paraformaldehyde for 30 minutes.

3. Wash in PBS.

4. Incubate in Block Solution (PBS + 10% Fetal Bovine Serum) for 12-16 hours.

    a. Block solution should be changed three to four times every few hours and then left overnight. Additional changes can be done if necessary.

5. Add primary antibody in block solution. Incubate overnight at 4ºC.

    a. A higher concentration of primary antibody is recommended for cells present in a 3D BD PuraMatrix sample compared to that used for cells in 2D culture. Determine the optimal concentration by titrating the antibody.

    b. Process one sample **without primary antibody** as a negative control to assess background staining.

    c. Use sufficient volume to completely soak the 3D gel.

6. Wash in block solution at least four times (two hours per wash).

7. Add secondary antibody in block solution.

   a. As described in step 5a above, a higher concentration of secondary antibody is recommended for cells in BD™ PuraMatrix™.

   b. Incubate for four hours.

8. Wash again in PBS or block solution multiple times (four to six times) for at least one hour per wash.

9. Perform imaging analysis of the samples in the cell culture vessel directly. If necessary, carefully **remove the gel** from the vessel with spatula and place on a glass slide for microscopic analysis. If using a low percentage gel (e.g., <0.5%), use extreme care when transferring the gel.

MORPHOLOGICAL STAINING: RHODAMINE-PHALLOIDIN/DAPI

1. Gently remove media from insert or culture well.

2. Fix cells in gels in 4% paraformaldehyde for 20 minutes.

3. Gently remove fixative and discard.

4. Wash 1X with PBS

5. Add desired stain.

   a. We recommend 160 nM rhodamine-phalloidin in PBS, 300 µl per well or per insert.

   b. Cover and leave overnight.

   c. Add 1X DAPI for one hour and then rinse 2X with PBS.

6. Remove PBS and perform imaging analysis as described above for immunostaining.

I) Tips for *In Vivo* Delivery of Cells and/or Bioactive Molecules

- BD™ PuraMatrix™ can be readily handled using small or large bore needles and catheters. Small-bore needles may generate bubbles. To avoid the introduction of bubbles *in vivo*, use extreme care when filling and injecting samples with needles that are smaller than 20G.
- The material can be re-filtered with a 0.20 or 0.45 µm membrane filter prior to use.
- The material should be vortexed or sonicated prior to use to reduce viscosity.

While BD PuraMatrix can be injected as provided at low pH, some injection sites may not tolerate exposure to an acidic sample. Also, cells are sensitive to exposure to low pH. Therefore, the following protocol can be used to buffer and gel the material prior to injection into animals.

To prepare a 200 µl sample for injection, begin by aliquoting 50 µl of a cell/10% sucrose suspension into a small tube.

1. Add 50 µl of BD PuraMatrix and mix gently.

2. Immediately layer 100 µl of PBS (containing Ca^{2+} and Mg^{2+}) carefully over top of cell/hydrogel mixture.

3. Allow to sit at room temperature for 1 minute. Mix gently with a P200 pipet tip.

4. Using a 20-22 g needle, pull entire mixture into 1 cc syringe. Avoid air bubbles by slowly and carefully collecting the liquid.

5. A needle change is recommended for injection. In general, use a 30 g or 26 g needle for tissue or subcutaneous injections, respectively.

Notes:
- The volumes can be scaled according to requirements of the animal used and the injection site.
- The number of cells injected should be determined empirically according to the goals of the experiment.

Recommended Plating Volumes for BD™ PuraMatrix™ Peptide Hydrogel

	Growth Area (cm^2)	Volume Per Well (µl)
BD Falcon™ Cell Culture Plate		
6-well	9.60	1200
24-well	2.00	250
96-well	0.32	50

	Growth Area (cm^2)	Volume Per Insert (µl)
BD Falcon™ Cell Culture Inserts		
6-well	4.20	700
24-well	0.30	50
96-well	0.08	14

Note: The final volumes of BD PuraMatrix for plating (BD PuraMatrix/cell/sucrose mixture) should be doubled for cell encapsulation.

STABILITY

Stable for a minimum of three months from date of shipment when stored at 4-30ºC.

TECHNICAL SUPPORT

For technical assistance, contact Technical Support at:
tel: 877.232.8995 or 978.901.7389; fax: 978.901.7491; e-mail: labware@bd.com

CUSTOMER SERVICE

To place an order in the U.S., contact Customer Service at:
tel: 877.232.8995 fax: 800.325.9637 or 858.812.8889

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