

## Liver Biomatrix Coated Plates and Dishes

Liver Biomatrix is a novel matrix isolated from decellularized liver tissue. PhoenixSongs' proprietary process retains the liver matrix biochemistry leaving the matrix scaffold intact. Following the decellularization process, the matrix scaffold is reduced to  $\mu\text{m}$  sized particles in suspension which is coated onto multi-well plates and dishes. Biomatrix coated plates and dishes provide the ultimate microenvironment for maintaining functional hepatocytes in culture longer than on Collagen I. In addition, stem cells committed to the endodermal lineage differentiate into functional hepatocytes within a few days maintaining function for greater than 4 weeks in culture.

### Storage of Liver Biomatrix Coated Plates and Dishes

1. Store Liver Biomatrix coated plates and dishes at 4°C until ready to use

### Preparing Liver Biomatrix Coated Plates and Dishes For Receiving Primary Hepatocytes

1. Prepare Hepatocyte Maintenance media (Cat.11004-250) according to product insert
2. Remove the biomatrix coated plate from the package aseptically
3. For best results, rehydrate the biomatrix overnight. If there are time constraints, minimal rehydration time is at least 2 hours prior to use.

**CAUTION:** When adding liquid media to the well or dish, slowly disperse the media down the side of the well or dish.

**DO NOT** deliver liquid media directly onto the biomatrix coating as this could force some biomatrix particles to release from the plate leaving a hole in the biomatrix layer.

4. Add Hepatocyte Maintenance Medium to each well or plate and place in humidified incubator. Refer to Table 1 for volume.
5. When ready to add cells, remove the rehydration media, wash once with Hepatocyte Maintenance Medium taking care not to dispense media directly onto the biomatrix coating,

**CAUTION:** When aspirating liquid from the biomatrix coated plate/well or dish, slowly lower the aspiration pipette tip to the media surface taking care not to touch the bottom of the biomatrix coating on plate/well or dish.

**DO NOT** touch the biomatrix with aspiration pipette tip as this will result in aspiration of biomatrix particles that are in direct contact to the tip.

6. Add cell suspension in Hepatocyte Maintenance Media. Refer to Table 1 for volume and plating densities.

**NOTE:** Ensure that the cell suspension is homogenous when adding to the biomatrix coated plate. The cells will settle onto the biomatrix coating within 10 minutes so after adding the cells let the plates or dishes set for 10 minutes before moving them into the incubator.

## Preparing Liver Biomatrix Coated Plates and Dishes For Receiving Hepatoblasts or Stem Cells

1. Prepare Kubota's StemCell Growth Media (cat.# 11001-250) for stem cells of endodermal lineage or Kubota's Hepatoblast Growth Media (cat.# 11002-250 for lineage committed hepatoblasts according to product inserts.
2. Remove the biomatrix coated plate or dish from the package aseptically and place in safety cabinet.
3. For best results, rehydrate the biomatrix overnight. If there are time constraints, minimal rehydration time is at least 2 hours prior to use.

**CAUTION:** When adding liquid media to the well or dish, slowly disperse the media down the side of the well.

**DO NOT** deliver liquid media directly onto the biomatrix coating as this could force some biomatrix particles to release from the plate leaving a hole in the biomatrix layer.

4. Add Kubota's StemCell or Hepatoblast Growth Media to each well and place plate in humidified incubator. Refer to Table 1 for volume.
5. When ready to add cells, remove the rehydration media, wash once with Kubota's StemCell or Hepatoblast Growth Media taking care not to dispense media directly onto the biomatrix coating.

**CAUTION:** When aspirating liquid from the biomatrix coated plate/well or dish, slowly lower the aspiration pipette tip to the media surface taking care not to touch the biomatrix coating.

**DO NOT** touch the biomatrix with aspiration pipette tip as this will result in aspiration of biomatrix particles that are in direct contact to the tip.

6. Add cell suspension in Kubota's StemCell or Hepatoblast Growth Media. Refer to Table 1 for volume and plating densities.

NOTE: Ensure that the cell suspension is homogenous when adding to the biomatrix coated plate. The cells will settle onto the biomatrix coating within 10 minutes so after adding the cells let the plates set for 10 minutes before moving them into the incubator.

7. The next day remove the media from each well and feed with Hepatic Differentiation Media.
8. On day 4 post plating, remove the differentiation media and replace with Hepatocyte Maintenance Media.

**Table 1. Hepatic cells should be plated at confluence approximately  $2.00 \times 10^5$  cells/cm<sup>2</sup>**

Dish/Flask Size	Growth Area (cm <sup>2</sup> )	Plating Volume (ml)	Maximum Volume (ml)	Stem Cells per well	Hepatocytes per well
6-Well	9.6	2	3	$1.00-1.50 \times 10^6$	$1.50 \times 10^6$
24-Well	2.0	0.5	1.5	$3.00-4.00 \times 10^5$	$4.00 \times 10^5$
96-Well	.32	0.1	.2	$4.00-5.00 \times 10^4$	$5.0 \times 10^4$
100mm	58	6	10	$5.0-6.0 \times 10^6$	$9.00-10.0 \times 10^6$

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