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Liver Biomatrix Suspension

Liver Biomatrix Suspension is a novel new matrix isolated from decellularized liver tissue. The PhoenixSongs proprietary patent-pending process retains the liver matrix biochemistry leaving the matrix scaffold intact. Following the decellulariziation process, the matrix scaffold is reduced to µm sized particles in suspension which is used for coating multi-well plates. Biomatrix coated plates provide the ultimate microenvironment for maintaining functional hepatocytes longer than when plated 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.

Liver Biomatrix Suspension (Catalog #: 12005-012)

1. One vial frozen Liver Biomatrix Suspension

Storage of Liver Biomatrix Suspension

1. Store Liver Biomatrix Suspension Pack in -80°C freezer until ready to use

Preparing Liver Biomatrix for Coating Plates

Liver Biomatrix suspension is easy to work with but requires adherence to coating instructions for successful coating of plates. Improper handling of the Biomatrix suspension can result in clumping of the biomatrix material and uneven coating of the plates. After coating plates with Biomatrix, it is essential for the plates to remain undisturbed for a minimum of 2 hours preferable overnight.

1. Determine the number of plates you want to coat (see Table 1),
2. Aseptically remove the plates from the packaging and place on the flat level surface in a biological safety cabinet or laminar flow hood. Organize the plates so that you can add Biomatrix suspension without moving the plates.
3. Have pipettor, pipettes and reagent reservoir ready for use
4. Thaw the vial of Liver Biomatrix Suspension by rapid thaw method in 37°C water-bath. Submerse the vial up to the cap in the water-bath and continually agitate in water until completely thawed – should take a couple minutes.
    a. Slow thawing can result in clumping of biomatrix material
5. Coat plates immediately because allowing suspension to set for any length of time will result in clumping of the biomatrix material and uneven coating.

Table 1. Giga-Matrix™ plating volumes for multi-well plates.

Plate Size	Volume/well	Volume/Plate	Plates/Bottle
384-well	30ul	12.0ml	1
96-well	50ul	5.0ml	2
24-well	300ul	7.5ml	1
12-well	800ul	10ml	1
6-well	1.5ml	9ml	1

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Coating Plates with Liver Biomatrix Suspension

1. Transfer appropriate volume of biomatrix suspension into each well using a multi-channel pipettor where appropriate. Refer to Table 1 for plating volume.
    a. Ensure that the suspension is evenly distributed across well – tap plate if necessary but don't remove from flat surface of hood.
2. Let plates remain undisturbed for a minimum of 4 hours allowing biomatrix material to settle to the bottom of the well. Best to coat plates first thing in the morning.
    a. This step is critical for even coating of the well with biomatrix particles.
3. At the end of the day or after 4 hours remove solution from each well
    a. Handle plates carefully so matrix coating isn't disturbed
    b. Tilt plate toward you without removing plate edge from hood surface. This stabilizes plate to prevent jerky movements that may make biomatrix come off the plate
4. Using suction apparatus with fine tip pipettes aspirate solution from each well. DO NOT TOUCH MATRIX SURFACE WITH PIPETTE!!!
    a. Gently lay plate back down, leave lid off and allow to dry completely over night.
        i. Moving plate while wet disturbs the biomatrix coating which will result in an uneven coating of the well.
5. When biomatrix coating is completely dry replace lid and examine for quality
6. Coated plates may be stored in 4°C refrigerator for up to 1 year.

Preparing Liver Biomatrix For Receiving Primary Hepatocytes

1. Prepare Hepatocyte Maintenance media 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, slowly disperse the media down the side of the well as illustrated in Figure 1.
    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 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 Hepatocyte Maintenance taking care not to dispense media directly onto the biomatrix coating,

    CAUTION: When aspirating liquid from the biomatrix coated plate/well, slowly lower the aspiration pipette tip to the media surface taking care not to touch the bottom of the plate/well. **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 set for 10 minutes before moving them into the incubator.

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Preparing Liver Biomatrix For Receiving Stem/Progenitor Cells from Endodermal Lineage

1. Prepare Kubota's StemCell Growth media 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, slowly disperse the media down the side of the well as illustrated in Figure 1.
   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 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 Growth Media taking care not to dispense media directly onto the biomatrix coating.

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

Table 2. Plating densities and media volume for plating hepatocytes and stem cells **(~1.88 x 10^5 cells/cm^2 for hepatocytes and ~1.2 x 10^5 cells/cm^2 for stem cells) on biomatrix.**

Dish/Flask Size	Growth Area (cm^2)	Plating Volume (ml)	Maximum Volume (ml)	Stem Cells per well	Hepatocytes per well
384-Well	0.05	0.05	0.10	6.00×10^3	9.40×10^3
96-Well	0.32	0.10	0.20	4.00×10^4	4.88×10^4
24-Well	2.0	0.50	1.50	2.40×10^5	3.76×10^5
12-Well	3.5	1.00	2.00	4.20×10^5	6.58×10^5
6-Well	9.6	2.00	3.00	1.15×10^6	1.80×10^6

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