

STEMmatrix™ Spike-In

Defined, pre-coating-free matrix for serum-free, feeder-free maintenance of human pluripotent stem cells

Catalog #100-2230 1 Kit



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Product Description

STEMmatrix™ Spike-In is a defined cell culture matrix that supports the maintenance of human pluripotent stem cells (hPSCs) under serum- and feeder-free conditions. After diluting, STEMmatrix™ Spike-In can be added directly to TeSR™ maintenance medium when seeding hPSCs, and does not require additional pre-coating steps or incubation time. STEMmatrix™ Spike-In contains Laminin-511 fragments and has been optimized for use with ReLeSR™ (Catalog #100-0483) and ACCUTASE™ (Catalog #07920) for the dissociation of hPSCs. This matrix is compatible with mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), TeSR™-E8™ (Catalog #05990), TeSR™-AOF (Catalog #100-0401), or eTeSR™ (Catalog #100-1215).

Component Storage and Stability

The following components are sold as a complete kit (Catalog #100-2230) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMmatrix™ Spike-In Supplement	300-1330	2 x 175 µg (0.35 mL/vial)	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMmatrix™ Spike-In Buffer	100-2260	2 x 2.8 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique to prepare cultureware with diluted STEMmatrix™ Spike-In.

A. PREPARATION OF DILUTED STEMmatrix™ SPIKE-IN

1. Add STEMmatrix™ Spike-In Supplement to STEMmatrix™ Spike-In Buffer at a 1 in 9 dilution using a polyethylene terephthalate (PETG) or polypropylene (PP) tube or bottle.

For example, to prepare 3.15 mL of diluted STEMmatrix™ Spike-In, add 350 µL (i.e. 175 µg) of STEMmatrix™ Spike-In Supplement to 2.8 mL of STEMmatrix™ Spike-In Buffer.

2. Swirl the vial to mix the diluted STEMmatrix™ Spike-In. Do not vortex.

NOTE: If not used immediately, store diluted STEMmatrix™ Spike-In at 2 - 8°C for up to 6 weeks. Do not exceed the shelf life of the individual components. Do not freeze.

B. PREPARING CULTUREWARE WITH DILUTED STEMmatrix™ SPIKE-IN

Use diluted STEMmatrix™ Spike-In at a ratio of 4.7 µL per cm² surface area of the cultureware. See Table 1 for surface areas and recommended volumes of diluted STEMmatrix™ Spike-In for various cultureware.

Table 1. Recommended Volumes of Diluted STEMmatrix™ Spike-In for Various Cultureware

CULTUREWARE	SURFACE AREA (cm ²)	VOLUME OF DILUTED STEMmatrix™ SPIKE-IN
6-well plate	9.6	45 µL/well
12-well plate	3.5	16.4 µL/well
24-well plate	1.9	8.9 µL/well
48-well plate	1.1	5.2 µL/well
96-well plate	0.32	1.5 µL/well
T-25 cm ² flask	25	117 µL/flask
T-75 cm ² flask	75	352 µL/flask

For complete instructions on culturing hPSCs, refer to the Technical Manuals: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #1000005505), mTeSR™ Plus (Document #1000007757), TeSR™-E8™ (Document #1000005516), TeSR™-AOF (Document #1000008160), or eTeSR™ (Document #1000019963). These documents are available at www.stemcell.com, or contact us to request a copy.

Preparing Cultureware with Diluted STEMmatrix™ Spike-In

The following protocol is for preparing one well of a 6-well tissue culture-treated plate with diluted STEMmatrix™ Spike-In. If using other cultureware, adjust volumes accordingly (see Table 1).

NOTE: Only use tissue culture-treated cultureware (e.g. Falcon® 6-Well Flat-Bottom Plate, Tissue Culture Treated; Catalog #38016) with diluted STEMmatrix™ Spike-In.

1. Add 2 mL of complete TeSR™ maintenance medium (e.g. mTeSR™1, mTeSR™ Plus, TeSR™-E8, TeSR™-AOF, or eTeSR™) to one well of a 6-well tissue culture-treated plate.
2. Add 45 µL of diluted STEMmatrix™ Spike-In (i.e. 4.7 µL per cm² x 9.6 cm² x 1 well) to the well. Gently swirl the plate to mix.
3. Seed hPSCs as directed in a cell maintenance protocol of choice.

NOTE: Diluted STEMmatrix™ Spike-In may be added to cultureware before or after seeding hPSCs.

Batch Protocol For Culturing with Diluted STEMmatrix™ Spike-In

For preparing multiple wells or plates, a large volume of TeSR™ maintenance medium + diluted STEMmatrix™ Spike-In (medium-matrix solution) can be prepared before dispensing into cultureware. The following example is for preparing 12.27 mL of medium-matrix solution to dispense into 6 wells of a 6-well tissue culture-treated plate. If preparing additional wells or using other cultureware, adjust volumes accordingly (see Table 1).

1. Add 12 mL of complete TeSR™ maintenance medium (i.e. 2 mL x 6 wells) to a PETG or PP tube.
2. Add 270 µL of diluted STEMmatrix™ Spike-In (i.e. 4.7 µL per cm² x 9.6 cm² x 6 wells) to the same tube. Gently invert or pipette the medium-matrix solution to mix.
3. Evenly distribute the entire volume of medium-matrix solution (~2 mL/well) across 6 wells of a 6-well tissue culture-treated plate.
4. Seed hPSCs as directed in a cell maintenance protocol of choice.

NOTE: hPSCs may be added to medium-matrix solution before dispensing into cultureware.

C. PASSAGING hPSCs AS AGGREGATES

hPSC cultures maintained with STEMmatrix™ Spike-In can be passaged as aggregates using ReLeSR™ (Catalog #100-0483). The following instructions are for passaging cells from TeSR™ cultures grown in 6-well plates. If using other cultureware, adjust volumes accordingly.

NOTE: Gentle Cell Dissociation Reagent is not recommended for dissociating cultures maintained with STEMmatrix™ Spike-In.

1. Aliquot a sufficient volume of TeSR™ medium and warm to room temperature (15 - 25°C). Do not warm medium in a water bath.
2. Wash cells with 1 mL/well of D-PBS (Without Ca⁺⁺ and Mg⁺⁺; Catalog #37350) and aspirate.
3. Add 1 mL/well of ReLeSR™ and aspirate to completely remove the ReLeSR™ immediately or within 1 minute (if working with multiple wells), so that colonies are exposed to only the residual liquid.
4. Incubate at 37°C for **5 - 12 minutes**.

NOTE: Optimal dissociation time may vary depending on the cell line used; when passaging a cell line with ReLeSR™ for the first time, the optimal dissociation time should be determined by observing for the moment when gaps begin to appear between cells located on the edges of the colonies under magnification. For additional information on dissociating hPSCs using ReLeSR™, refer to the Product Information Sheet (PIS; Document #1000008733).

5. Add 1 mL/well of TeSR™ medium by hovering the serological pipette above the well.
6. Detach the colonies by holding the plate with one hand and using the other hand to firmly tap the side of the plate for approximately 30 - 60 seconds.
7. Transfer the detached cell aggregates to a 15 mL conical tube (e.g. Catalog #38009) using a 5 mL serological pipette (e.g. Catalog #38003). Cell aggregates should be appropriately sized for plating (mean aggregate size of approximately 50 - 200 µm).
NOTE: If you wish to plate cell aggregates directly from your passaged well (i.e. without transferring into a tube), pipette the aggregate mixture up and down once using a 5 mL pipette to break up any large aggregates that may still be present.
8. Perform clump count and seed the cell aggregate mixture at the desired density into complete TeSR™ medium containing diluted STEMmatrix™ Spike-In as described in sections A and B.
9. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cell aggregates. Do not disturb the plate for 24 hours.

D. HARVESTING SINGLE-CELL SUSPENSIONS OF hPSCs

hPSC cultures maintained with STEMmatrix™ Spike-In can be passaged as single cells using ACCUTASE™ (Catalog #07920) to prepare single-cell suspensions for downstream applications (e.g. flow cytometry).

NOTE: Gentle Cell Dissociation Reagent is not recommended for dissociating cultures maintained with STEMmatrix™ Spike-In.

The following are instructions for preparing a single-cell suspension from TeSR™ cultures grown in 6-well plates. If using other cultureware, adjust volumes accordingly. Cultures should be harvested and used for downstream applications at the time they would normally be ready for passaging.

1. Warm TeSR™ medium and ACCUTASE™ to room temperature (15 - 25°C) before use.

NOTE: Do not warm TeSR™ medium in a 37°C water bath. For complete instructions on thawing and storing ACCUTASE™, refer to the PIS (Document #1000000377).

2. Use a microscope to visually identify regions of differentiation and remove by scraping with a pipette tip or by aspiration.
3. Wash cells with 1 mL/well of D-PBS (Without Ca⁺⁺ and Mg⁺⁺) and aspirate.
4. Add 1 mL/well of ACCUTASE™ and incubate at 37°C for **5 - 10 minutes**.

NOTE: Incubation time may vary when using different cell lines or other cell passaging reagents. Dissociation should be monitored under the microscope until the optimal time is determined.

5. Add 2 mL/well of TeSR™ medium. Using a 1 mL pipettor, gently wash the cells from the surface of the plate by spraying the solution directly onto the colonies.
6. Harvest cells by pipetting up and down with either a serological pipette or a 1 mL pipettor to ensure a single-cell suspension and transfer cells to a 15 mL conical tube. If needed, rinse wells with an additional 2 - 4 mL of TeSR™ medium and add the rinse to the tube containing the cells.
7. Centrifuge cells at 300 x g for 5 minutes.
8. Resuspend cells in appropriate medium or buffer for desired downstream applications.

E. PASSAGING eTeSR™ CULTURES

STEMmatrix™ Spike-In can be used to support the routine single-cell passaging of hPSCs in eTeSR™ (Catalog #100-1215).

To maintain eTeSR™ cultures using STEMmatrix™ Spike-In, passage cells as directed in the eTeSR™ PIS (Document #1000019962) with the protocol adjustments listed below. Volumes are listed for 6-well plates; if using alternate cultureware, adjust volumes according to surface area.

- Dissociate hPSCs using **1 mL/well** of ACCUTASE® or TrypLE™ Express Enzyme (Thermo Fisher Scientific® Catalog #12605010) and incubate at 37°C for **5 - 10 minutes**.

NOTE: Incubation time may vary when using different cell lines or passaging reagents. Dissociation should be monitored under the microscope until the optimal time is determined.

- After counting cells, plate the single-cell suspension at the desired density into Single-Cell Plating Medium containing diluted STEMmatrix™ Spike-In, as described in sections A and B.

For complete instructions on maintaining hPSCs in eTeSR™, refer to the Technical Manual: Expansion of Human Pluripotent Stem Cells in eTeSR™ (Document #1000019963), available at www.stemcell.com, or contact us to request a copy.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow, or contact us at techsupport@stemcell.com.

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