STEMium®

Serum-free and feeder-free medium for human pluripotent stem cells

Reagents include:

Material	Catalog #	Volume	Storage
STEMium® basal medium	5801	500 mL	4- 8°C
StemGS® 50X growth supplement	5852	10 mL	-20°C
StemDS® cell dissociation solution	5803	100 mL	4°C

Please thoroughly read the entire brochure and contact us if you have questions before proceeding.

Medium Preparation

All work must be done using proper sterile tissue culture techniques and equipments.

1. Thaw StemGS® 50X supplement (Catalog #5852) at room temperature (15-25°C) or overnight at 4°C.

Alternatively, thawed StemGS® 50X supplement can be immediately aliquoted and stored in -20°C for up to 3 months. Thawed StemGS® should be used within 1 day to prepare complete medium. Repeated freezing and thawing of StemGS® is not recommended.

2. Add the entire 10 mL of thawed StemGS® 50X to 490 mL STEMium® basal medium (Catalog # 5801) and mix well. This complete STEMium® is stable for up to 2 weeks at 4°C.

Avoid frequent warming of complete STEMium® by aliquoting the complete STEMium® into working volumes.

Adapting human embryonic stem cells (hESCs) to STEMium®

All work must be done using proper sterile tissue culture techniques and equipments.

Prior to culturing human embryonic stem cells (hESCs) in STEMium®, it is recommended that a backup stock of your cells should be cryo-preserved.

Advantages of using STEMium®

Consistent formulation No batch to batch variability due to our unique serum-free, xeno-free formulation.

Convenient Does not require feeder cells, conditioned media or additional growth factors. Minimal medium adaptation is required.

Low bFGF concentration Unlike other commercial human pluripotent stem cell media, complete STEMium® contains low concentration of bFGF

Easy Subculturing Convenient method using our StemDS® cell dissociation solution. No additional scraping tools are required.

Feeder-free Human embryonic stem cells cultured in STEMium® do not require feeder cells and should be used in conjunction with BD Matrigel™.

1. Passage cells using previous culture conditions and medium on day 1.

2. Aspirate medium and rinse with 4mL STEMium® on day 2. Cells can now be cultured in STEMium® from here on.

Passaging hESCs cultured in STEMium®

All work must be done using proper sterile tissue culture techniques and equipments.

StemDS® is a specially formulated human embryonic stem cell dissociation solution. This gentle and effective reagent should be used in conjunction with STEMium® to passage cells, as other passaging methods have not been validated. Cells should be subcultured when they remain in log phase and cell colonies are nearly touching (70-75% confluent) with less than 20% of differentiated cells.

1. Coat tissue culture plate or dish with BD Matrigel™ (Catalog #354277) according to the manufacturer's instructions and warm to room temperature.

Do not allow BD Matrigel™ to evaporate before usage. Avoid scratching the BD Matrigel™ coated surface.

2. Warm complete STEMium®, StemDS® and DPBS (Catalog #0303) to room temperature.

3. If present, remove regions of differentiation by aspiration or scraping with a pipette tip. Refer to comment below.

4. Aspirate old medium from hESCs and wash cells 2-3 times with DPBS (2 mL per well of a six-well plate or 5mL per 10cm dish).

5. Aspirate DPBS and gently add 1 mL StemDS® per well or 3 mL StemDS® per 10 cm dish. Incubate at 37°C for 5-6 minutes.

Colonies should remain adherent after incubation when viewed under microscope. Refer to appendix 2.

6. Aspirate StemDS® completely.

No need to wash cells with DPBS after StemDS® incubation.

7. Pipette 2-4 mL STEMium® and dislodge cells from plate.

Best if a 5 mL serological pipette is used to loosen the colonies. If needed, use P1000 tip to gently resuspend any large visible clumps. Optimal splitting ratio is 1:3 to 1:4.

8. Gently disperse the appropriate amount of STEMium® containing the cell aggregates into the BD Matrigel™-coated plate or dish after aspirating out the BD Matrigel™ solution.

9. Fill the remaining vessel with appropriate volume of STEMium® and distribute cells evenly by rocking the plate several times. Incubate cells in 37°C 5% CO_2 with 95% humidity incubator.

Use a minimum of 3mL STEMium® per well of a six-well plate and 15mL STEMium® per 10cm dish.

General Comments

* Medium should be changed on a daily basis due to the rapid growth rate that hESCs exhibit.

* Differentiation is characterized by different cell morphologies, when visualized under a microscope. Mark areas containing differentiated cells for removal prior to cell passaging or cryo-preservation. Remove these regions by manual dissection, scraping or aspiration. The differentiation areas should be less than 20% of the entire well or dish.

* Morphology of hESCs grown in STEMium® will be different compared to other media (refer to Appendix 1). Cells are more tightly packed in the center of each colony with defined borders, as compared to hESCs grown in MEF-conditioned medium.

* StemDS® incubation period will vary depending on the cell line and cell density at the time of splitting. Therefore, this incubation time should be empirically determined by the individual user.

* This product is intended for research use only.

Your detailed feedback is important in helping us improve our products. Please take a moment and contact us with your comments.

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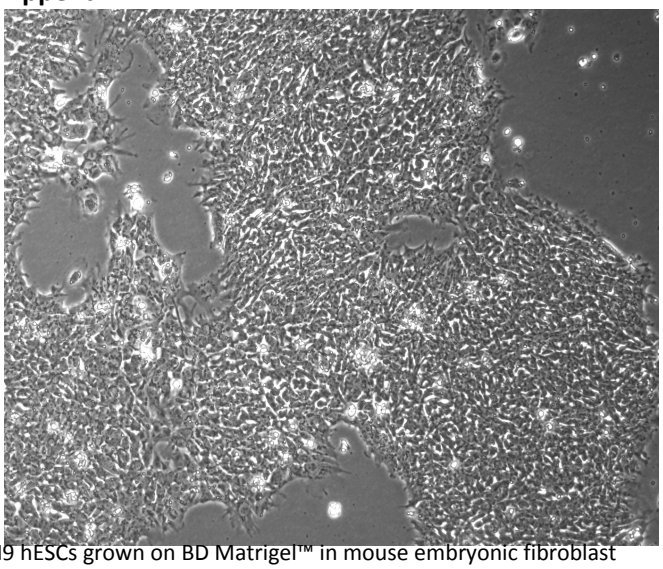
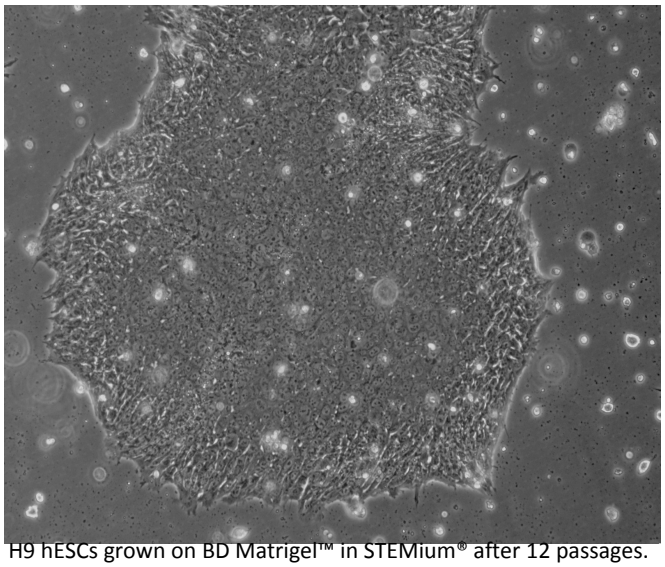
Appendix 1

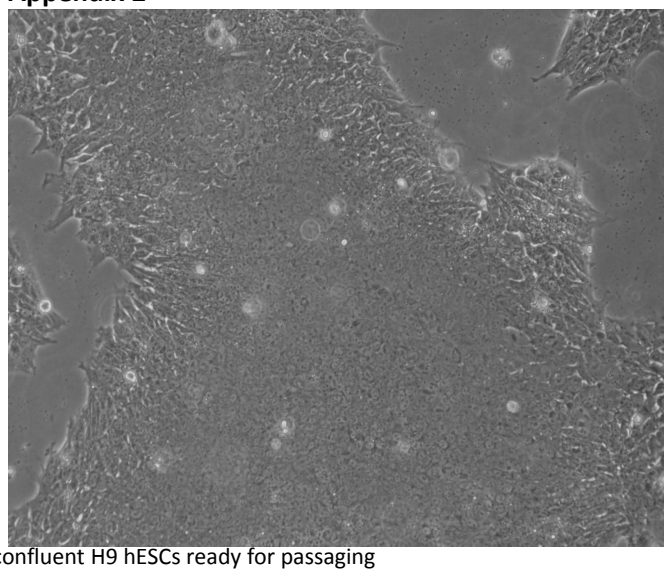


H9 hESCs grown on BD Matrigel™ in mouse embryonic fibroblast conditioned medium.

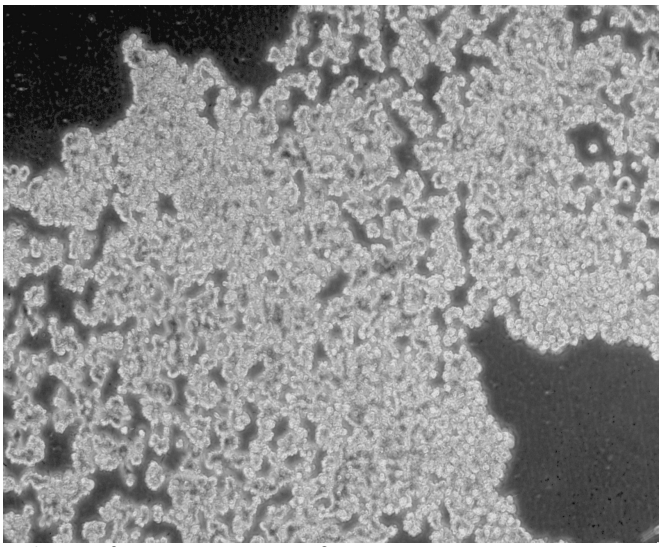


H9 hESCs grown on BD Matrigel™ in STEMium® after 12 passages.

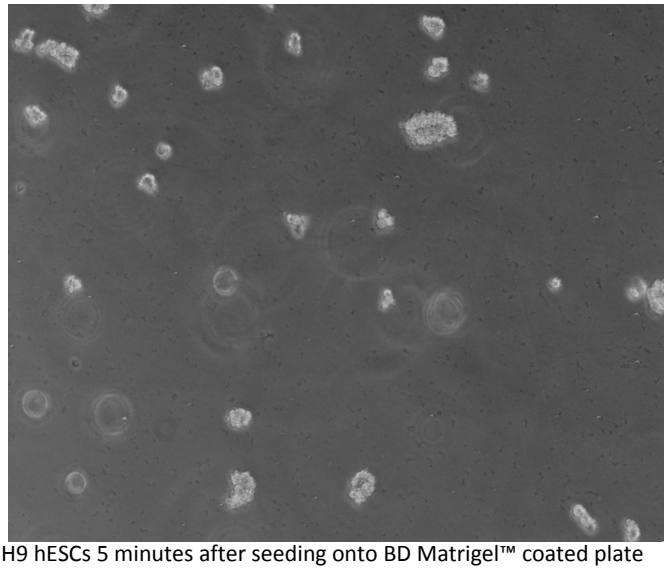
Appendix 2



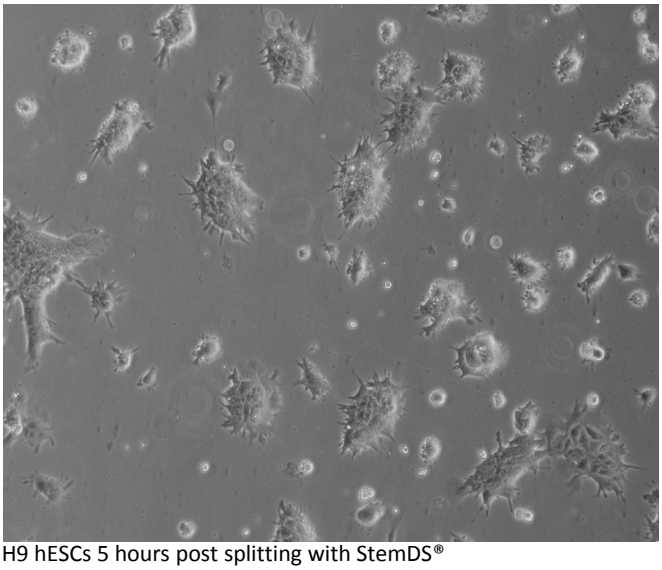
confluent H9 hESCs ready for passaging



H9 hESCs after 6 minute StemDS® treatment.



H9 hESCs 5 minutes after seeding onto BD Matrigel™ coated plate



H9 hESCs 5 hours post splitting with StemDS®

ScienCell™ Research Laboratories