PEPROTECH®
Manufacturer of Quality Cytokine Products

Instructional Manual

PeproGrow Animal-Free Low Protein hESC Medium

Maintenance Medium for hESCs and hiPSCs

ANIMAL-FREE Low Protein

Product Use Limitations: Not for human use.

Made in the United States.

Feeder-Free Culture Techniques for Pluripotent Stem Cells using PeproGrow Animal-Free Low Protein hESC Medium

A. Introduction

PeproGrow Animal-Free Low Protein hESC Medium is an animal component-free, serum- and phenol red-free medium of a complete, chemically-defined formulation designed for feeder-free maintenance and expansion of both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs). This medium formulation is best used with PeproTech's Animal-Free Human Vitronectin Matrix as a surface-coating reagent; however, it can also be used with Corning Matrigel® or Synthemax® II-SC. This medium is intended for the culturing of hESCs and iPSCs in the undifferentiated, pluripotent state (Nanog+/Lin28+/Tra-1-60+ or SSEA4+/Oct4+), and demonstrates less than 15% spontaneous differentiation as indicated by immunofluorescent staining. Similar to the PeproGrow hESC Medium (Catalog Number BM-hESC), this proprietary formulation includes relevant growth factors, such as FGF2 (FGF-basic), but does not contain the insulin found in the majority of other hESC/iPSC media currently available on the market. This medium does not contain albumin so researchers may need to adjust laboratory techniques that were developed for albumin-containing media. PeproGrow Animal-Free Low Protein hESC Medium, which was designed and developed by PeproTech in collaboration with the Stem Cell Training Course at Rutgers University, is supplied as a 100mL, or 500mL, bottle of basal medium and a separate, lyophilized growth factor component. Additional companion products, such as the Animal-Free Human Vitronectin Matrix and Buffer Kit, and the Cell Passaging/Non-Enzymatic Detachment Buffer, are available separately.

NOTE: Cell culture media accommodation may be required depending on the cell type used (refer to page 4 under "E. Suggested Protocols for Cell Culture Accommodation"). Enzymatic cell passaging and freezing methods should not be used for this cell culture medium, and the PBS/EDTA reagents are recommended (refer to page 3 under "General recommendations" for additional information).

B. Materials and Reagents

1. PeproGrow Animal-Free Low Protein hESC Medium:

Kit/Components	Catalog Number	Size
PeproGrow Animal-Free Low Protein hESC Medium Kit	**AF-LP-hESC-500**	
Low Protein Basal Medium	AF-BM-LP-hESC-500	500mL
Animal-Free Growth Factor Component	AF-GF-hESC-500	Vial for 500mL Basal Medium
PeproGrow Animal-Free Low Protein hESC Medium Kit	**AF-LP-hESC-100**	
Low Protein Basal Medium	AF-BM-LP-hESC-100	100mL
Animal-Free Growth Factor Component	AF-GF-hESC-100	Vial for 100mL Basal Medium

2. Refer to the appendix for additional materials and reagents, including the product listing of PeproTech's Animal-Free stem cell media and companion products.

C. Preparation of Medium and Growth Factor Component

1. Reconstitute the lyophilized Animal-Free Growth Factor Component with 500µL of sterile cell-culture grade water.
2. If using the entire contents of the basal medium within two weeks, then aseptically add the 500µL of reconstituted Animal-Free Growth Factor Component to the basal medium, and mix well by swirling. If not immediately using the entire contents of the basal medium, then aseptically transfer the necessary volume of basal medium into a sterile polycarbonate bottle or a conical bottom polypropylene tube. Add the necessary proportion of reconstituted Animal-Free Growth Factor component to the basal medium, and mix well by swirling. Filtration is not necessary if prepared aseptically.
3. Label the bottle with both the date of mixture and the newly calculated expiration date (2 weeks from date of mixture). Store at 2°C to 8°C.
4. Remove only the necessary amount of medium for immediate use and warm to room temperature (RT) prior to feeding cells. Do not warm the medium in a water bath. If necessary due to time constraints, remove a working volume of media from the complete medium bottle and warm in a 37°C water bath for no more than 5 minutes.

Storage/Stability: Keep medium in the dark.

Product Form	Temperature	Storage Time
Liquid Basal Medium	2°C to 8°C; Keep in the dark.	6 months
Liquid Basal Medium after preparation	2°C to 8°C; Keep in the dark.	2 weeks
Animal-Free Growth Factor Component (lyophilized)	-20°C to -80°C 2°C to 8°C	5 years 6 months

Avoid repeated freeze-thaw cycles.

General recommendations:

- **Recommended Coating for Cell Culture Ware:** It is recommended, but not required, to first use Corning Matrigel® when adapting to PeproGrow Animal-Free Low Protein hESC Medium; however, other matrices have also been tested and shown to function well with PeproGrow Animal-Free Low Protein hESC Medium. PeproTech's Animal-Free Recombinant Human Vitronectin Matrix and Buffer Kit (Catalog Number AF-VMB-220) is recommended to be used at 5µg/mL, and performs optimally with standard cell dishes or CellBind® dishes (Corning). Refer to "D. Preparation of Animal-Free Human Vitronectin Matrix-Coated Plates/Dishes."
- **Please Note:** Enzymatic methods should not be used with this medium for cell culture passaging or freezing procedures. It is recommended to use PeproTech's osmotically compatible Cell Passaging/Non-Enzymatic Detachment Buffer (Catalog Number CPD-125), which contains PBS, HEPES, and EDTA.
- Refer to the Appendix for additional reagents and protocols.

D. Preparation of Animal-Free Human Vitronectin Matrix-Coated Cell Plates/Dishes

NOTE: When using Animal-Free Human Vitronectin Matrix-coated cell plates/dishes, enzymes cannot be used during cell culture steps. It is recommended to use PeproTech's Cell Passaging/Non-Enzymatic Detachment Buffer (Catalog Number CPD-125), which contains PBS, HEPES and EDTA.

1. Reconstitute the Animal-Free Human Vitronectin Matrix according to the product sheet instructions using PeproTech's osmotically compatible PBS + Kolliphor P 188 (Catalog Number AF-VMB-220 for the Animal-Free Human Vitronectin Matrix and Buffer Kit).
2. Coat the cell culture plates/dishes with 5µg/mL of Animal-Free Human Vitronectin Matrix using the recommended cell culture well volumes for Corning plastic dishes as listed in the chart. For other manufacturer's cell dishes, adjust the volume in relation to the surface area, and ensure the plastic surface area is completely covered.

Dish Size	Surface Area (cm2)	Volume (mL)
6 cm dish	21	2.5
10 cm dish	55	5
1 well of a 6-well dish	9.5	1
1 well of a 12-well dish	3.8	0.5
1 well of a 24-well dish	1.9	0.3

3. If using the dish on the same day, then place the dish in a warm humidified cell culture incubator for 2 hours. Otherwise, wrap each dish with Parafilm and store at 4°C on a flat surface for up to one week. When storing at 4°C, a slightly larger volume may be required later if evaporation/condensation is observed on the cell culture dish lid.
4. After the coating incubation time, aspirate the Animal-Free Human Vitronectin Matrix solution and immediately add 1-2mL of PeproGrow Animal-Free Low Protein hESC Medium (pre-conditioning step) into a well of a 6-well dish. Adjust volumes for other sized dishes/plates. Do not allow the dish surface to dry or the coating matrix may not function as expected.
5. Return the dish to the incubator to warm the media. Use the dish the same day.

E. Suggested Protocols for Cell Culture Medium Accommodation

NOTE: PeproGrow Animal-Free Low Protein hESC Medium can be substituted in place of the researcher's currently used medium. If an acute or "No accommodation" method (see below) does not function as expected, then the additional accommodation protocols below may be used. From laboratory observations, when changing the current laboratory medium to the PeproGrow Animal-Free Low Protein hESC Medium, the H9 cell line and hiPSCs, require "No accommodation" or a "Short accommodation," while the H1 hESCs require a "Long accommodation" adaptation. In the rare occasion that these recommended methods do not work for the pluripotent cell line, then use the "Long accommodation" combined with a gradual increasing percentage of the PeproGrow Animal-Free Low Protein hESC Medium over the course of one week, until the only medium used is the PeproGrow Animal-Free Low Protein hESC Medium (i.e. use media ratios of 80:20, 60:40, 40:60, 20:80, until 100% changeover to the PeproGrow Animal-Free Low Protein hESC Medium is achieved).

a. No accommodation:
    1. Passage near-confluent cultures using the PBS/EDTA method onto Animal-Free Human Vitronectin Matrix-coated dishes using the PeproGrow Animal-Free Low Protein hESC Medium.
    2. Culture the cells as recommended in the Instructional Manual.

b. Short accommodation:
    1. Passage cells onto Animal-Free Human Vitronectin Matrix-coated dishes with the current media using the PBS/EDTA method.
    2. Allow the cells to grow for 2-4 days, and change the medium to the PeproGrow Animal-Free Low Protein hESC Medium.
    3. Culture the cells as recommended in the Instructional Manual.

c. Long accommodation:
    1. Plate cells on Corning Matrigel®-coated dishes in the current medium.
    2. After 2-4 days, once the cells have begun to grow rapidly, change the medium to the PeproGrow Animal-Free Low Protein hESC Medium.
    3. Allow the cells to grow to normal confluence.
    4. Passage the cells using the PBS/EDTA method onto Animal-Free Human Vitronectin Matrix-coated dishes.

F. Maintenance of Pluripotent Stem Cells

NOTE: Cultures may appear sparse within a day of thawing or splitting; however, hESCs/iPSCs grow very rapidly after 3-4 days in culture. Please note that colonies may vary morphologically in comparison to a high protein cell culture medium, whereas the cells appear round, spread less, and exhibit a 3rd dimension growth in height. Maintain cells by changing the media on a daily basis, with the exception of accepting one "double volume" feed over the weekend. When supplementing with a "double volume," it is recommended to passage the cells on a Thursday or Friday, so that the double feed occurs when the cells are at a lower density (e.g. cells can receive 4mL/well on a Friday to avoid the need for a feed on a Saturday, with a feeding on Sunday). Alternatively, cells can be passaged Friday and plated into 4mL of medium, and then fed on a Sunday.

EXAMPLE: 6 well dish

1. Check every well for growth, and monitor cultures for bacterial or fungal microorganisms.
2. Aspirate each well to remove old media, and add 2mL of RT media into each well.

G. Thawing Cryopreserved Pluripotent Stem Cells

NOTE: Cells of an ultra-frozen sample should be thawed to 37°C slowly and consistently in order to avoid temperature shock and shear stress. The use of cold and cool solutions allows for the gradual introduction of a newly thawed, and still cold, sample to warmer environments in a controlled manner. Once the cryopreserved sample has been thawed, it is important to dilute out the DMSO as soon as possible and to transfer the sample to a suitable culture vessel. In addition, cells grown in the PeproGrow Animal-Free Low Protein hESC Medium on PeproTech's Animal-Free Human Vitronectin Matrix, and non-enzymatically cyropreserved using PBS/EDTA, may be thawed into the PeproGrow Animal-Free Low Protein hESC Medium and then plated onto the Animal-Free Human Vitronectin Matrix-coated substrate. This procedure has been streamlined below so that it can be performed within 15 minutes.

1. Place a 15mL conical tube in a rack (pre-cooled Biocision CoolRack™ 15mL) in a Biosafety Level 2 (BL2) Laminar Flow hood, and fill with 9mL of cold media.
2. Remove a vial of cells (hESC or iPSC) from liquid nitrogen (LN_2) vapor phase storage tank, place in floater rack, and transfer to 37°C water bath.

   WARNING: Instructions for the majority of cryovials caution against storage of samples in the liquid phase of LN_2 due to the possibility of LN_2 accumulation in the vial, which can cause an explosion during rapid thawing. To reduce the risk of an explosion, vials should be transferred to a -80°C freezer the night before use to allow for the slow evaporation of any LN_2 that may have leaked into the vial.
3. Do not shake the vial. To ensure that the average temperature in the vial remains cool, observe occasionally and remove the vial when only a small amount of ice remains.
4. Spray the vial generously with 70% ethanol, wipe, and transfer to an appropriate rack into a sterile environment in a BL2 laminar flow hood. To prevent further temperature fluctuations, it is highly recommended to use a pre-cooled Biocision CoolRack® XT CFT24. Most freeze racks (Biocision, Corning) have locking grooves to unscrew the top of the ampoule; however, the majority of these racks do not secure the vial vertically into the rack. Be careful not to spill the cells if working with multiple vials.
5. Using a disposable, sterile, plastic transfer pipette, remove 1mL of media from the 15mL tube previously prepared in step 1, and slowly back-fill the cryovial with gentle stirring. Alternatively, use a 1-2mL serological pipette, or a 1000µL sized micropipette.
6. Remove contents of the vial to a 15mL conical tube.
7. **OPTIONAL:** Rinse the vial with a second round of 2mL diluted cells/media from the 15mL tube using the same transfer pipette.
8. If there are large clumps of cells, then allow larger aggregates of cells to settle at 4°C in a precooled Biocision CoolRack™ 15mL tube on ice (or placed in the refrigerator) for 5-10 minutes, skip the following centrifugation step, and proceed to step 11.
9. Remove the dishes coated with Animal-Free Human Vitronectin Matrix (or other appropriate matrix) from the incubator and place in a laminar flow hood to equilibrate to RT.
10. Centrifuge the cells for 2-5 minutes at 100 x g at 4°C. Alternatively, if using a Biocision CoolRack™ (or ice) and the medium is still cool to the touch, then spin the tube at RT.
11. Remove the supernatant and gently resuspend the cells in 2mL of cool medium. Keep the tube in hand to warm for 30 seconds or, alternatively, place the tube in the rack for 1-2 minutes.

12. Place the cell suspension into an appropriate number of Animal-Free Human Vitronectin Matrix-coated culture-ware wells. Use RT medium to perform further dilutions for cells. For example, if the vial contains 1 confluent well of a 6-well dish, then plate in 1-3 wells. Adjust the volume of cells to 3mL, and dispense 1mL per well in a dish already containing 1mL media per well.

    TIP: When using 6-well dishes only, plate cells out by running the pipette tip around the rim of the well while slowly dispensing the cell solution. It is recommend to use a 5mL glass serological pipette for this step, as the bore is smooth compared to the plastic version and may offer less shearing stress to the cell clumps.

    Not plating on a 6-well dish? While it is recommended to passage cells at least once before using them for analytical purposes (immunostaining, flow cytometry, western blot analysis, Q-RT-PCR, etc.), cells can be plated directly on other sized vessels at this point. Please be aware that the freeze/thaw process can introduce a relatively noticeable amount of differentiation. For an indirect immunofluorescence technique, it is recommended to use 24-well dishes. For passing cells onto a 96-well-size dish, or any dish with more than 96 wells per dish, it is recommended to use PBS/EDTA.

H. Passaging Pluripotent Stem Cells Cultures Using PBS/EDTA

NOTE: When passaging cells into a 96-well dish (or any small vessel with high surface tension such as a 4-well or 8-well chamber slide), it is recommended to remove the cells from the culture dish using PBS/EDTA. This treatment dissociates the colonies into small clumps and will ensure a more even spreading of cells in the well. To ensure even spreading and to overcome surface tension, the cells should be spin-seeded at 80-120 x g for 2-5 minutes, onto the culture dish using appropriate plate carriers. Without spin-seeding, cells will tend to clump towards the center of the well. If plate holders for the appropriate centrifuge are not available, then minimal volumes should be used to ensure a faster attachment. Cells passaged in the morning should be attached by early afternoon, at which time the low volume of media can be gently topped off with incubator-warmed, pre-equilibrated medium.

1. Aspirate the media from the cell culture(s). Add 1-2mL PBS/EDTA.
2. Place the plate in the incubator and observe after 3 minutes; returning the plate to the incubator as needed. After 5 minutes, the colonies that were previously "smooth" should have many holes (resembling lacey Swiss cheese), and a much brighter phase contrast around many of the cells.

    PROTOCOL TIP: If the cells have not yet begun to detach, then quickly and gently aspirate the PBS/EDTA. Immediately and somewhat forcefully squirt the desired volume of medium onto the cells using a circular motion from the center to the outside of the well. Proceed to step 7 following centrifugation as the cell suspension can be plated immediately.
3. Once the cells have begun to detach from the plate, gently squirt 3-4mL of culture medium, or suitable base medium, onto each well in a circular pattern from the outside towards the middle. This will wash all cells from the plate.
4. Gently transfer the cell suspension to a 15mL or 50mL conical tube. A 50mL size tube is highly recommended, as it can be difficult to remove the media/PBS/EDTA solution from a 15mL conical tube following centrifugation.
5. Centrifuge for 2-5 minutes at 120 x g at RT.
6. Aspirate the supernatant and gently add the appropriate amount of medium for the given split volume. For example, if splitting 1 well 1:6, then add 6mL of medium, and with a 5mL glass serological pipette, gently triturate once to disaggregate cells into a "sandy-water" consistency.

WARNING: Do not over triturate the cells at this point as it can lead to poor results.

7. Gently swirl the material in the well, and take up the needed split volume into a 5mL serological, or a wide-bore 1000µL pipette depending on the seeding volume. For example, if splitting the well 1:4 into a 4mL volume, then 1mL should be added per well, while a 1:8 split would use 0.5mL/well. A 1000µL pipette is recommended for the plating of any volumes below 500µL. However, the cell volume should be aspirated and dispensed slowly to avoid further shearing of the aggregates.
8. Transfer the portion to a new well/dish by distributing volume around the rim of the well. Cells will naturally gravitate towards the center of the wells during incubation; this technique leads to the even distribution of aggregates. This works well for 6-well dishes, but not for dishes of other dimensions. If cells are dense and the split volume is 3mL, then cells should be plated 1:6 to 1:12 using 0.5 to 0.25mL of the cell suspension.
9. If the colonies are at an optimal density, then the cells can be split every 5-7 days using 1:6 to 1:12 splits (i.e. the aggregates from 1 well of a 6-well plate can be plated in 6-10 wells). If the colonies are too dense or too sparse, then the split ratio should be adjusted accordingly. Passaging cells either too early, or too late, may result in decreased attachment or increased differentiation, respectively. Please note that these guidelines are based on the growth characteristics of the H1 hESC and two Rutgers Stem Cell Training Course iPSC lines, and results may vary between different cell lines and laboratories. Ensure that newly-seeded colonies are evenly distributed on the new Animal-Free Human Vitronectin Matrix-coated plate as failure to do so may also result in increased differentiation in culture.

I. Freezing Pluripotent Stem Cells with PBS/EDTA

NOTE: Cryopreservation methods are similar to cell passaging; however, it is highly recommended not to centrifuge the cells in order to remove the PBS/EDTA. Please refer to the protocol tip with step 2. This step removes the PBS/EDTA prior to the colonies lifting off the surface, and allows the cells to gently settle and cool to 4°C over a 15 minute time interval. While using this method, a large quantity of cell plates/dishes can be passaged, permitting the previously dissociated cultures to settle at 4°C.

1. Aspirate the medium from the cell culture, and add 1-2mL PBS/EDTA.
2. Place the plate in the incubator and observe after 3 minutes; returning the plate to the incubator as needed. After 5 minutes, the colonies that were previously "smooth" should have many holes (resembling Swiss cheese), and a much brighter phase contrast around many of the cells.

   PROTOCOL TIP: If the cells have not yet begun to detach, then quickly and gently aspirate the PBS/EDTA. Immediately and somewhat forcefully, squirt the desired volume of media onto the cells using a circular motion from the center to the outside of the well. Proceed to step 4.
3. Once the cells have begun to detach from the plate, gently squirt 2-4mL of culture media, or suitable base media, onto each well in a circular pattern from the outside towards the middle. This will wash all the cells from the plate.
4. For the PBS/EDTA non-centrifuge method, collect cells from multiple wells in a 15mL tube, and place the tube into a Biocision CoolRack 15mL, or place the tube on ice. Allow the cell clumps to settle for 5-15 minutes. If centrifuging the cells instead, then proceed to step 5. Otherwise, proceed to step 6.
5. Centrifuge the cells for 2-5 minutes at 100 x g at 4°C.
6. Aspirate the supernatant and add appropriate amount of cool media for the given volume (e.g. for one well, add 0.5mL media).
7. Add equal volume of 2x the freeze medium, and mix gently by slow inversion.

Refer to the Appendix "Preparation of 2X Defined-Humanized Freeze Medium (10% HSA)" for this medium's protocol.

8. Place 1mL of cells into a cryovial.
9. Place the cryo-vials in a Biocision CoolCell®, or suitable alternative (e.g. Mr. Frosty), and store at -80°C. Transfer to LN_2 the following day.

Appendix–Reagents and Materials

PeproTech Stem Cell Culture Media Products

Kit/Components	Catalog Number	Size
PeproGrow Animal-Free hESC Medium Kit	**AF-R-hESC-500**	
Basal Medium	AF-BM-hESC-R-500	500mL
Animal-Free Growth Factor Component	AF-GF-hESC-500	Vial for 500mL Basal Medium
PeproGrow Animal-Free hESC Medium Kit	**AF-R-hESC-100**	
Basal Medium	AF-BM-hESC-R-100	100mL
Animal-Free Growth Factor Component	AF-GF-hESC-100	Vial for 100mL Basal Medium
PeproGrow Animal-Free Low Protein hESC Medium Kit	**AF-LP-hESC-500**	
Low Protein Basal Medium	AF-BM-LP-hESC-500	500mL
Animal-Free Growth Factor Component	AF-GF-hESC-500	Vial for 500mL Basal Medium
PeproGrow Animal-Free Low Protein hESC Medium Kit	**AF-LP-hESC-100**	
Low Protein Basal Medium	AF-BM-LP-hESC-100	100mL
Animal-Free Growth Factor Component	AF-GF-hESC-100	Vial for 100mL Basal Medium

Companion Product/Components	Catalog Number	Size
Animal-Free Human Vitronectin Matrix and Buffer Kit Kit	**AF-VMB-220**	
Animal-Free Human Vitronectin Matrix		500µg
PBS + Kolliphor P 188		220mL
Cell Passaging/Non Enzymatic Detachment Buffer (Buffer contains PBS+HEPES+EDTA)	CPD-125	125mL -

Reagents and Materials for Cell Culture

1. PeproGrow Animal-Free Low Protein hESC Medium (PeproTech, AF-LP-hESC-500 for the 500mL size, or AF-LP-hESC-100 for the 100mL size); Refer to "C. Preparation of Media and Growth Factor Component" for instructions.
2. DMEM/F12, 340 mOsm or equivalent
3. Phosphate buffered saline (PBS)/EDTA 0.5mM, pH 7.4, 340mOsm (or PBS/EGTA)
4. Phosphate buffered saline, 340mOsm

5. Cell culture grade sterile water (ddH_2O)
6. DMSO (Sigma, D2650)
7. Human serum albumin (Sigma, A5843; powder)
8. Trehalose (Sigma,T0167)
9. HEPES (Corning, 25-060-CI)
10. Corning Matrigel® (Corning, 354277; hESC qualified or equivalent)
11. Cell culture plastics (many vendors, for 6 well dishes Corning, BD Biosciences, and Sarstedt can be used)

Reagents and Materials for Immunostaining

1. Oct4 (1:1000-2000, monoclonal rabbit antibody, Life Technologies, A13998)
2. Tra-1-60 (1:200-300, mouse IgM, Life Technologies, 411000)
3. Nanog (1:250; mouse IgG1, Sigma, N3038, clone NNG-811)
4. Lin28 (1:500; monoclonal rabbit antibody, Abcam, ab124765)
5. DAPI (5mg/mL stock, dilute 1:10000 to 500ng/mL in PBS for use; Sigma, D9542)
6. Goat-anti-mouse-IgM-AlexaFluor 594 (1:500-1000, Life Technologies)
7. Goat-anti-mouse-IgG1-AlexaFluor 488 (1:500, Life Technologies, A21121)
8. Goat-anti-rabbit-AlexaFluor 647 (1:500-1000, Life Technologies)
9. ProLong® Gold mounting medium (Life Technologies)
10. Glass coverslips (12mm)
11. Fine forceps
12. PBS
13. ddH_2O
14. Normal goat serum (EMD Millipore, S26-100ML)
15. Triton X-100 (diluted to 10% in PBS)
16. Bovine serum albumin (low endotoxin, Fatty Acid, IgG Free)
17. Blocking solution (10% goat serum, 1% BSA, 0.1% Triton X-100, 20mM HEPES in PBS)
18. Antibody Solution (0.5x Blocking Solution in PBS)

Reagents and Materials for Flow Cytometry

NOTE: When using iPSCs that have been reprogrammed using Oct4, it is highly recommended to substitute the Oct4 antibody with the Nanog antibody. Inclusion of a viability dye is optional. The use of BD Perm/Wash™ buffer (BD Biosciences, 554723) is crucial when using Oct4 or Nanog antibodies.

1. Human and Mouse Pluripotent Stem Cell Analysis Kit (BD Biosciences)
2. Nanog-PerCP-Cy™5.5 (BD Biosciences, 562259)

Preparation of 2x Defined – Humanized Freeze Medium (10% HSA)

1. Measure 20mL 25% HSA in DMEM/F12.
2. Dissolve 5 grams trehalose into 12-15 mL DMEM/F12.
3. Add 10mL DMSO.
4. Fill the volume to 50mL using DMEM/F12.
5. Filter using a 0.2 micron SFCA filter unit.
6. Aliquot into 15mL conical tubes and freeze at -20°C until further use.

PeproTech, Inc • 5 Crescent Avenue • P.O. Box 275 • Rocky Hill, NJ 08553-0275
Tel: (800) 436-9910 • Fax: (609) 497-0321 E-mail: info@peprotech.com • sales@peprotech.com