Proteome Profiler™ Array

Human Pluripotent Stem Cell Array Kit

Catalog Number ARY010

For the parallel determination of the relative levels of human stem cell markers.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Analyzing the expression profiles of stem cell markers is helpful for understanding the differentiation mechanisms of human stem cells and developing disease treatments. The Human Pluripotent Stem Cell Array Kit is a rapid, sensitive, and economical tool to simultaneously detect the relative levels of expression of 15 stem cell markers without performing numerous individual immunoprecipitations and Western blots. Each antibody was carefully selected using cellular extracts prepared from cell lines known to express the target protein.

PRINCIPLE OF THE ASSAY

Capture and control antibodies have been spotted in duplicate on nitrocellulose membranes. Cellular extracts are diluted and incubated overnight with the Human Pluripotent Stem Cell Array. The array is washed to remove unbound proteins, followed by incubation with a cocktail of biotinylated detection antibodies. Streptavidin-HRP and chemiluminescent detection reagents are applied, and a signal is produced at each capture spot corresponding to the amount of protein bound. Refer to the Appendix for a list and coordinates of analytes and controls.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This kit should not be used beyond the expiration date on the kit label.
- **Do not mix or substitute reagents with those from other lots or sources. Substitution of some high intensity chemiluminescent reagents for Chemi Reagents 1 and 2 may cause either increased background or diminished signal depending on the reagent.**
- Any variation in sample handling, buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The Human Pluripotent Stem Cell Array membranes are validated for single use only.
- Always use gloved hands and flat-tipped tweezers to handle the membranes.
- Pick up the membranes from the edge on the side with the identification number avoiding the area with the printed antibodies.
- A thorough and consistent wash technique is essential for proper assay performance. Individual arrays should be washed in separate containers to minimize background. Wash Buffer should be removed completely from the membrane before proceeding to the next step.
- Do not allow the membrane to dry out. This will cause high background.
- Avoid microbial contamination of reagents and buffers.
- For a procedure demonstration video, please visit: www.RnDSystems.com/ProteomeProfilerVideo.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Pluripotent Stem Cell Array	893691	8 nitrocellulose membranes each containing 15 different anti-stem cell marker antibodies printed in duplicate.	Return unused membranes to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 3 months at 2-8 °C.*
Lysis Buffer 16	895935	21 mL of a non-denaturing buffered solution.	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 1	895477	21 mL of a buffered protein base with preservatives.	
Array Buffer 2 Concentrate (5X)	895478	21 mL of a concentrated buffered protein base with preservatives.	
Array Buffer 3	895008	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	3 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Detection Antibody Cocktail, Human Pluripotent Stem Cell Array	893690	1 vial of a biotinylated antibody cocktail; lyophilized	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	2.5 mL of stabilized hydrogen peroxide with preservative.	
Chemi Reagent 2	894288	2.5 mL of stabilized luminol with preservative.	
8-Well Rectangular Multi-dish	607591	Clear 8-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607675	1 transparency overlay template for coordinate reference.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)
- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- Plastic containers with the capacity to hold 50 mL (for washing the arrays)
- Plastic transparent sheet protector (trimmed to 10 cm x 12 cm and open on three sides)
  Plastic wrap
- Paper towels
- Absorbent lab wipes (KimWipes® or equivalent)
- Autoradiography cassette
- Film developer
- X-ray film (Kodak® BioMax™ Light-1, Catalog # 1788207) or equivalent
- Flat-tipped tweezers
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

PRECAUTIONS

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child. Do not handle until all safety precautions in the MSDS have been read and understood.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Since the Human Pluripotent Stem Cell Array detects relative expression levels of individual analytes, it is important to include appropriate control samples.

Note: *Sample amount may be empirically adjusted to attain optimal sensitivity with minimal background. Suggested starting range for cell lysates is 50-300 µg.*

Cell Lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding lysis buffer. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer 16 (prepared as described in the Reagent Preparation section). Pipette up and down to resuspend and rock the lysates gently at 2-8 °C for 30 minutes. Microcentrifuge at 14,000 x g for 5 minutes, and transfer the supernatant into a clean test tube. Quantitation of sample protein concentrations using a total protein assay is recommended. Assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Pluripotent Stem Cell Array - Eight nitrocellulose membranes each containing 15 different anti-stem cell marker antibodies printed in duplicate. **Handle the membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - Before use, reconstitute the Human Pluripotent Stem Cell Detection Antibody Cocktail in 200 µL of deionized or distilled water.

Lysis Buffer 16 - Add 10 µg/mL of Aprotinin, 10 µg/mL of Leupeptin, and 10 µg/mL of Pepstatin to the volume of lysis buffer required for cell lysate preparation. **Prepare fresh for each use.**

1X Array Buffer 2/3 - Dilute 1.8 mL of Array Buffer 2 Concentrate into 7.2 mL of Array Buffer 3. **Prepare fresh for each use.**

1X Wash Buffer - If crystals have formed in the concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

Chemi Reagent Mix - Chemi Reagents 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 0.5 mL of the resultant mixture is required per membrane.**

ARRAY PROCEDURE

Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipette 1.0 mL of Array Buffer 1 into each well of the 8-Well Multi-dish to be used. Array Buffer 1 serves as a block buffer.
3. Using flat-tip tweezers, remove each membrane to be used from between the protective sheets and place in a well of the 8-Well Multi-dish. The number on the membrane should be facing upward.

   Note: *Upon contact with Array Buffer 1, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*
4. Incubate for one hour on a rocking platform shaker. Orient the tray so that each membrane rocks end to end in its well.
5. While the arrays are blocking, prepare samples by adding up to 167 µL of lysate to 0.833 mL of Array Buffer 1. Adjust to a final volume of 1.0 mL with Lysis Buffer 16 as necessary. The maximum allowable lysate volume is 167 µL/array.
6. Aspirate Array Buffer 1 from the wells of the 8-Well Multi-dish and add the prepared sample. Place the lid on the 8-Well Multi-dish.
7. Incubate overnight at 2-8 °C on a rocking platform shaker.

   Note: *A shorter incubation time may be used if optimal sensitivity is not required.*
8. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 8-Well Multi-dish with deionized or distilled water and dry thoroughly.
9. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
10. For each array, dilute 20 µL of reconstituted Detection Antibody Cocktail to 1.0 mL with 1X Array Buffer 2/3. Pipette 1.0 mL per well of diluted Detection Antibody Cocktail into the 8-Well Multi-dish.
11. Carefully remove each array from its wash container. Allow excess Wash Buffer to drain from the array. Return the array to the 8-Well Multi-dish containing the diluted Detection Antibody Cocktail, and cover with the lid.
12. Incubate for 2 hours on a rocking platform shaker.
13. Wash each array as described in steps 8 and 9.
14. Dilute the Streptavidin-HRP in 1X Array Buffer 2/3 using the dilution factor on the vial label. Pipette 1.0 mL into each well of the 8-Well Multi-dish.

ARRAY PROCEDURE *CONTINUED*

15. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Return the array to the 8-Well Multi-dish containing the diluted Streptavidin-HRP, and cover it with the lid. Incubate for 30 minutes on a rocking platform shaker.

16. Wash each array as described in steps 8 and 9.

    Note: *Complete the remaining steps without interruption.*

17. Carefully remove each membrane from its wash container. Allow excess Wash Buffer to drain from the membrane by blotting the lower edge onto paper towels. Place each membrane on the bottom sheet of the plastic sheet protector with the identification number facing up.

18. Pipette 0.5 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

    Note: *Using less than 0.5 mL of Chemi Reagent Mix per membrane may result in incomplete membrane coverage.*

19. Carefully cover with the top sheet of the plastic sheet protector. Gently smooth out any air bubbles and ensure Chemi Reagent Mix is spread evenly to all corners of each membrane. Incubate for 1 minute.

20. Position paper towels on the top and sides of the plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.

21. Remove the top plastic sheet protector and carefully lay an absorbent lab wipe on top of the membranes to blot off any remaining Chemi Reagent Mix.

22. Leaving membranes on the bottom plastic sheet protector, cover the membranes with plastic wrap taking care to gently smooth out any air bubbles. Wrap the excess plastic wrap around the back of the sheet protector so that the membranes and sheet protector are completely wrapped.

23. Place the membranes with the identification numbers facing up in an autoradiography film cassette.

    Note: *Use an autoradiography cassette that is not used with radioactive isotope detection.*

24. Expose membranes to X-ray film for 1-10 minutes. Multiple exposure times are recommended.

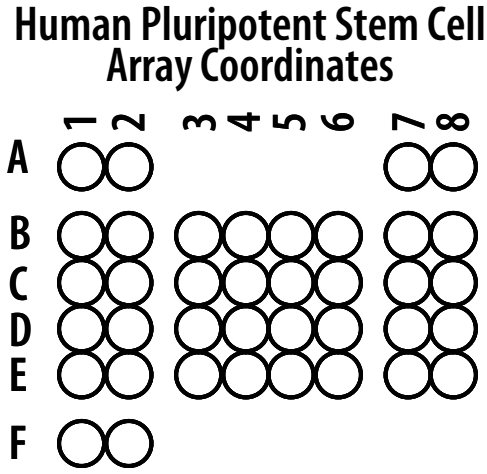
DATA ANALYSIS

The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. The location of controls and capture antibodies is listed in the Appendix.

Note: *Reference spots are included to align the transparency overlay template and to demonstrate that the array has been incubated with Streptavidin-HRP during the assay procedure.*

Pixel densities on developed X-ray film can be collected and analyzed using a transmission-mode scanner and image analysis software.

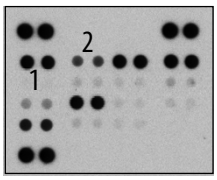
1. Create a template to analyze pixel density in each spot of the array.
2. Export signal values to a spreadsheet file for manipulation in a program such as Microsoft Excel.
3. Determine the average signal (pixel density) of the pair of duplicate spots representing each stem cell marker.
4. Subtract an averaged background signal from each spot. Use a signal from a clear area of the array or negative control spots as a background value.
5. Compare corresponding signals on different arrays to determine the relative change in cell stem cell marker levels between samples.

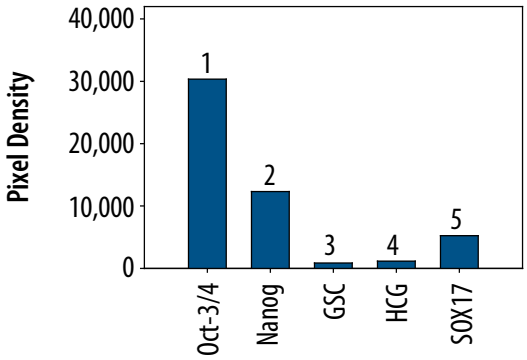


This image is not to scale. It is for coordinate reference only.
Please use the transparency overlay for analyte identification.

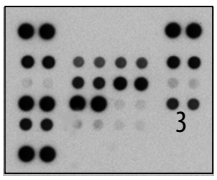
PROFILING STEM CELL MARKERS IN CELL LYSATES

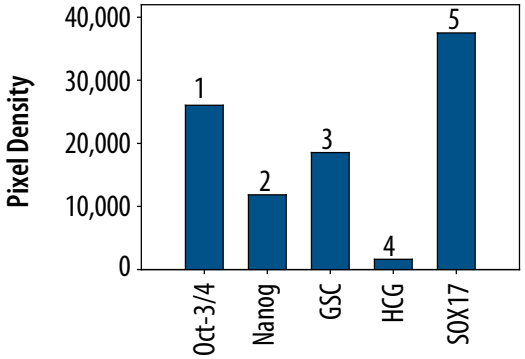
1A Undifferentiated



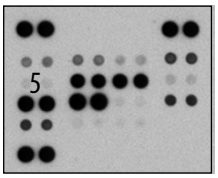


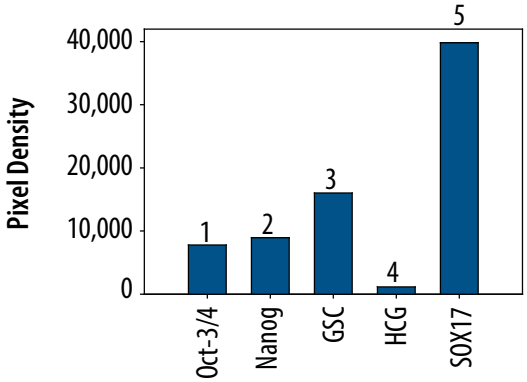
1B Mesendoderm



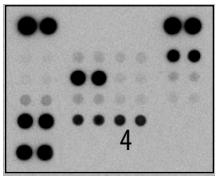


1C Endoderm





1D Trophectoderm



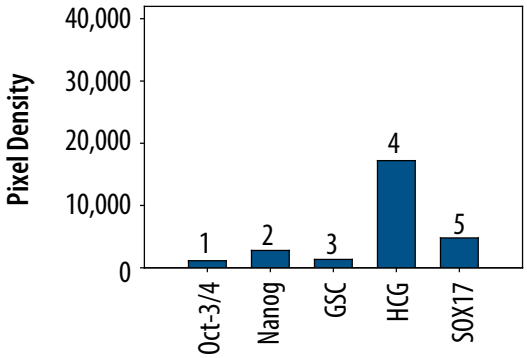


Figure 1A-1D: The Human Pluripotent Stem Cell Array detects multiple stem cell markers in differentiated BG01V cell lysates. 200 µg of cell lysate was run on each array. Data shown are from a 3 minute exposure to X-ray film.

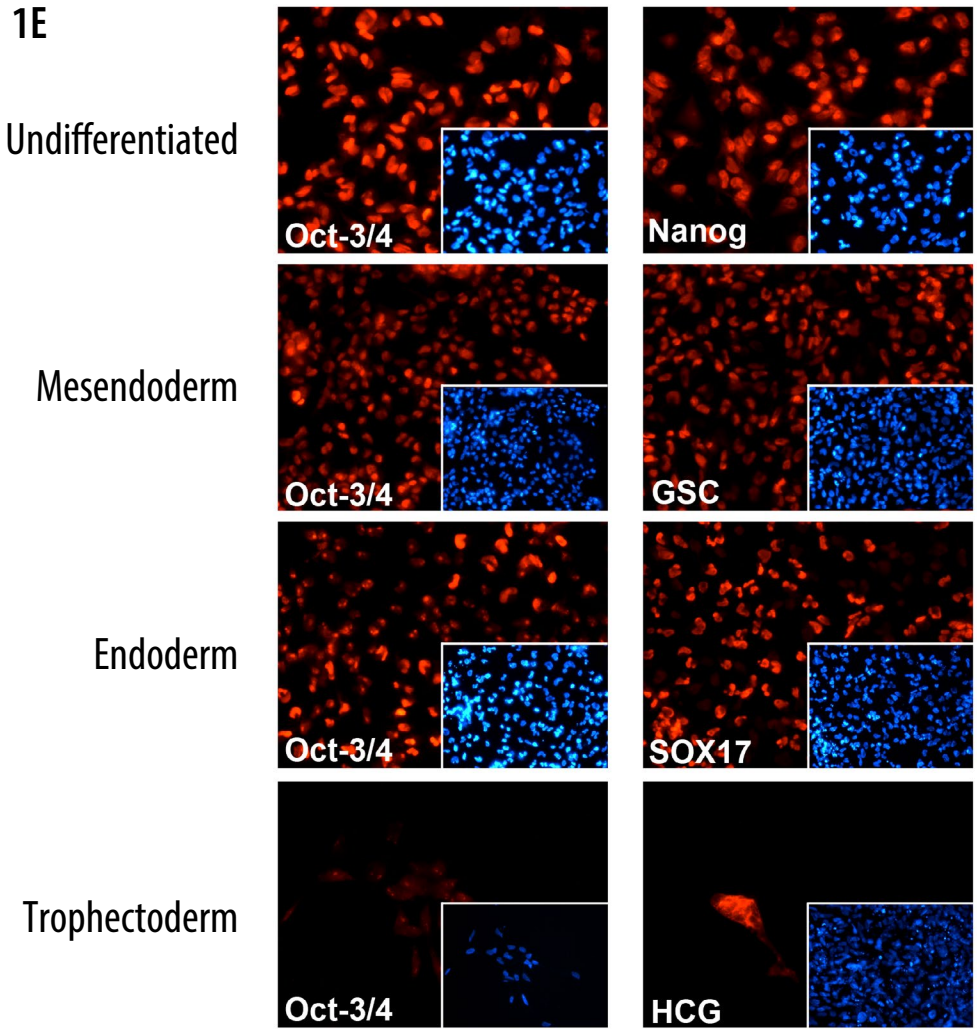
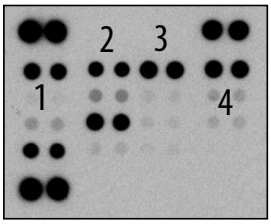


Figure 1E: Pluripotent Stem Cell Array data was confirmed by immunocytochemistry for each BG01V differentiation. Cells were stained with anti-human Oct-3/4 antibody (R&D Systems, Catalog # AF1759), anti-human Nanog antibody (R&D Systems, Catalog # AF1997), anti-human SOX17 antibody (R&D Systems, Catalog # AF1924), anti-human Goosecoid antibody (R&D Systems, Catalog # AF4086), or anti-human HCG antibody (R&D Systems, Catalog # MAB4169) as shown above. DAPI nuclear staining is shown in each image insert.

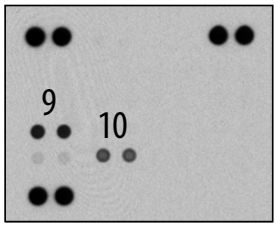
Extracts were prepared from BG01V hES cells grown under undifferentiated conditions in MEF Conditioned Media (R&D Systems, Catalog # AR005) supplemented with recombinant human (rh) FGF basic (R&D Systems, Catalog # 4114-TC). For mesendoderm differentiation, cells were grown in serum-free media in the presence of recombinant mouse (rm) Wnt-3a (R&D Systems, Catalog # 1324-WN) and recombinant human/mouse/rat Activin A (R&D Systems, Catalog # 338-AC) for two days. For endoderm differentiation, cells were first differentiated into mesendoderm as described above and subsquently grown in media containing only recombinant human/mouse/rat Activin A for two days. For trophectoderm differentiation, cells were grown in MEF Conditioned Media supplemented with rhFGF basic and rhBMP-4 (R&D Systems, Catalog # 314-BP) for 7 days.

PROFILING STEM CELL MARKERS IN SELECTED CELL LINES

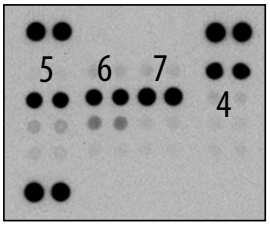
2A Ntera-2



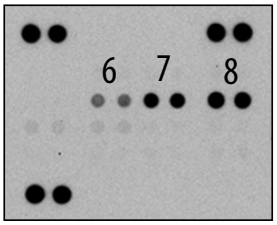
2B HUVEC



2C HepG2



2D β-TC-6



1	Oct-3/4
2	Nanog
3	SOX2
4	E-Cadherin
5	AFP
6	GATA-4
7	HNF-3β
8	PDX-1
9	SOX17
10	VEGF R2

Figure 2A-2D: The Human Pluripotent Stem Cell Array detects multiple protein markers in various cell lysates. 200 µg of cell lysate was run on each array. Data shown are from a 3 minute exposure to X-ray film.

SENSITIVITY

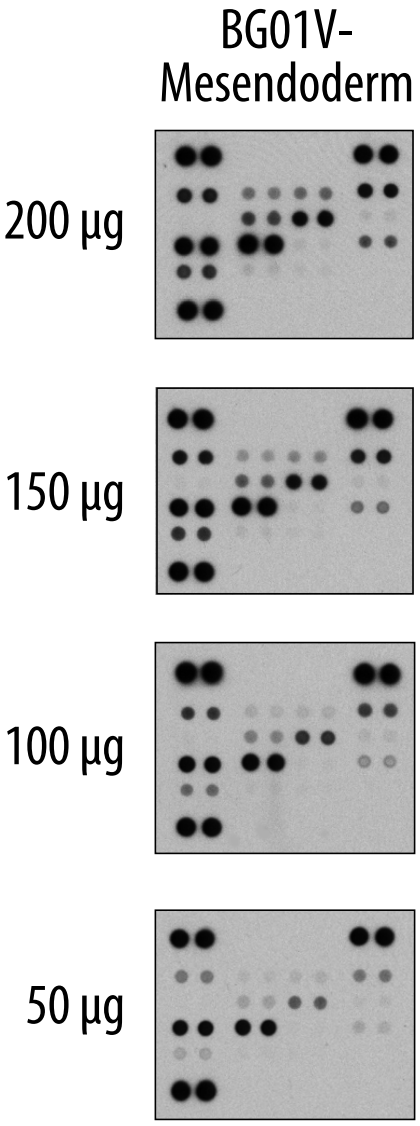


Figure 3: Signal intensities for stem cell markers may be modulated by the quantity of cell lysate incubated with the Human Pluripotent Stem Cell Array. Arrays were incubated with 50-200 µg of BG01V mesendoderm differentiated lysate as shown above. Data shown are from a 2 minute exposure to X-ray film.

APPENDIX

Refer to the table below for the Human Pluripotent Stem Cell Array coordinates.

Coordinate	Target/Control
A1, A2	Reference Spots
A7, A8	Reference Spots
B1, B2	Oct-3/4
B3, B4	Nanog
B5, B6	SOX2
B7, B8	E-Cadherin
C1, C2	α-Fetoprotein (AFP)
C3, C4	GATA-4
C5, C6	HNF-3β/FoxA2
C7, C8	PDX-1/IPF1
D1, D2	SOX17
D3, D4	Otx2
D5, D6	TP63/TP73L
D7, D8	Goosecoid (GSC)
E1, E2	Snail
E3, E4	VEGF R2/KDR/Flk-1
E5, E6	HCG
E7, E8	Negative Control (PBS)
F1, F2	Reference Spots

NOTES

NOTES