

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 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 the target capture antibodies.

TECHNICAL HINTS AND LIMITATIONS

- 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.
- Any variation in 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 membrane from the edge on the side with the identification number.
- A thorough and consistent wash technique is essential for proper assay performance. Individual arrays should be washed in separate containers to prevent high 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 recommended chemiluminescent detection reagents, see the Other Materials Required section. The use of some high intensity chemiluminescent reagents may cause either increased background or diminished signal depending on the reagent.

MATERIALS PROVIDED

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

Component	Part #	Storage Conditions	Amount Provided
Human Pluripotent Stem Cell Array	893691	2 - 8° C	8 membranes
Array Buffer 1	895477	2 - 8° C	1 vial (21 mL)
Array Buffer 2 Concentrate, 5X	895478	2 - 8° C	1 vial (21 mL)
Array Buffer 3	895008	2 - 8° C	1 vial (21 mL)
Lysis Buffer 16	895935	2 - 8° C	1 vial (21 mL)
Wash Buffer Concentrate, 25X	895003	2 - 8° C	3 vials (21 mL each)
Human Pluripotent Stem Cell Array Detection Antibody Cocktail	893690	2 - 8° C	1 vial
Streptavidin-HRP	890803	2 - 8° C	1 vial
8-Well Rectangular Multi-dish	607591	Room Temperature	1 dish
Transparency Overlay Template	607675	Room Temperature	1 template

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma, Catalog # A6279)
- Leupeptin (Sigma, Catalog # L8511)
- Pepstatin (Sigma, Catalog # P4265)
- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- Flat-tipped tweezers
- Rocking platform shaker
- Microcentrifuge
- Plastic containers with the capacity to hold 50 mL (for washing the arrays)
- Plastic transparent sheet protector
- Plastic wrap
- Chemiluminescent detection substrate (Pierce[®], Catalog # 32106 or Amersham, Catalog # RPN2132)
- Autoradiography cassette
- Film developer
- X-ray film (Kodak[®] BioMax[™] Light-1, Catalog # 1788207) or equivalent
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft[®] Excel

Pierce is a registered trademark of the Thermo Fisher Corporation.

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SAMPLE PREPARATION

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

Cell Lysates - Rinse cells with PBS and remove any remaining PBS before adding lysis buffer. Solubilize the cells at 1×10^7 cells/mL in Lysis Buffer 16 prepared with protease inhibitors. 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 supernate into a clean test tube. A total protein assay is recommended to determine the protein concentration of the cell lysate. The Human Pluripotent Stem Cell Array should be incubated with a quantity of lysate similar to that used for an immunoprecipitation or Western blot (50 - 300 µg). The maximum allowable lysate volume is 167 µL/array. Cell lysates should be used immediately or aliquoted and stored at $\leq -70^\circ$ C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

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 arrays only with gloved hands and flat-tipped tweezers.** After opening, reseal unused membranes in the foil pouch with desiccant and store at 2 - 8° C for up to 3 months.*

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Human Pluripotent Stem Cell Detection Antibody Cocktail in 200 µL of deionized or distilled water. Store at 2 - 8° C for up to 3 months after reconstitution.*

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

Array Buffer 1 - Ready for use. Store at 2 - 8° C for up to 3 months after initial 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 - Dilute 60 mL of 25X Wash Buffer Concentrate into 1440 mL of deionized or distilled water. Store at 2 - 8° C for up to 3 months after initial use.*

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

ARRAY PROTOCOL

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. For blocking each array, pipette 1.0 mL of Array Buffer 1 into each well of the 8-Well Multi-dish which will be used.
3. Using flat-tip tweezers, remove each array to be used from between the protective sheets.
4. Place one array into each well of the 8-Well Multi-dish and place the lid on the 8-Well Multi-dish. The array number should be facing upward.
Note: *Upon contact with Array Buffer 1, the blue dye will disappear from the spots. The capture antibodies are retained in their specific locations.*
5. Incubate the arrays in Array Buffer 1 for one hour on a rocking platform shaker. Orient the tray so that each array rocks end to end in its well.
6. In a separate tube, add the desired quantity 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 μ L/array.
7. Remove Array Buffer 1 from the 8-Well Multi-dish.
8. Add the diluted lysates and place the lid on the 8-Well Multi-dish.
9. Incubate overnight at 2 - 8° C on a rocking platform shaker.
Note: *Although sensitivity may be compromised, a 2 hour incubation may be done in place of the overnight incubation.*
10. Carefully remove each array 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.
11. Wash each array with 1X Wash Buffer by soaking for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
12. For each array, dilute 20 μ L 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.
13. 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.
14. Incubate for 2 hours on a rocking platform shaker.
15. Wash each array as described in steps 10 and 11.
16. 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.
17. 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.
18. Wash each array as described in steps 10 and 11.
19. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane. Place the membrane on a plastic sheet protector. Expose membranes to chemiluminescent reagents as directed by the manufacturer.
20. Cover the membranes with plastic wrap and expose to X-ray film for 30 seconds to 10 minutes. Multiple exposures are recommended.

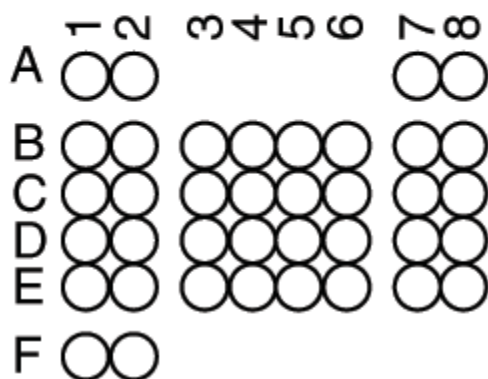
DATA ANALYSIS

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

Pluripotent Stem Cell Array data on developed X-ray film can be quantitated by scanning the film on a transmission-mode scanner and analyzing the array image file using 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 stem cell marker levels between samples.

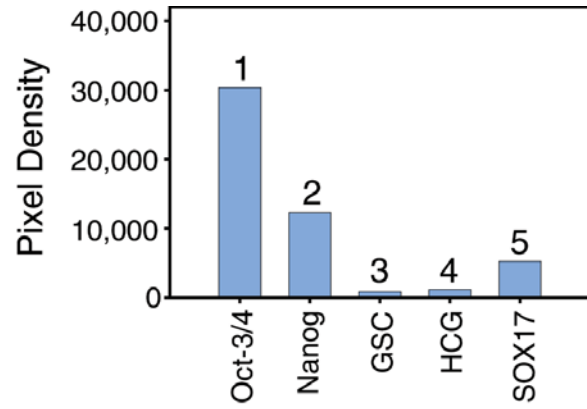
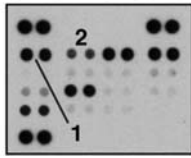
Human Pluripotent Stem Cell Array Coordinates



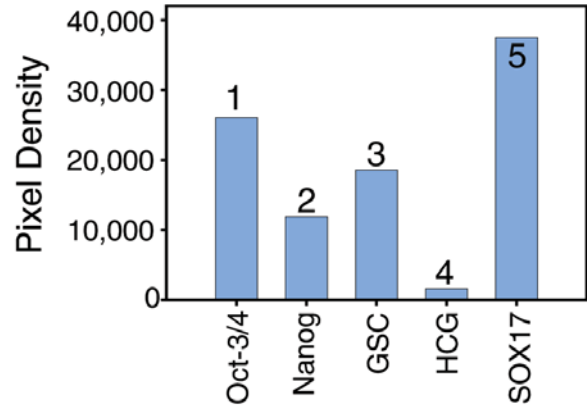
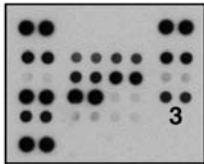
This image is not to scale; it is for coordinate reference only.
Please use the transparency overlay for analyte identification.
See the Appendix on page 11.

PROFILING STEM CELL MARKERS IN BG01V EXTRACTS

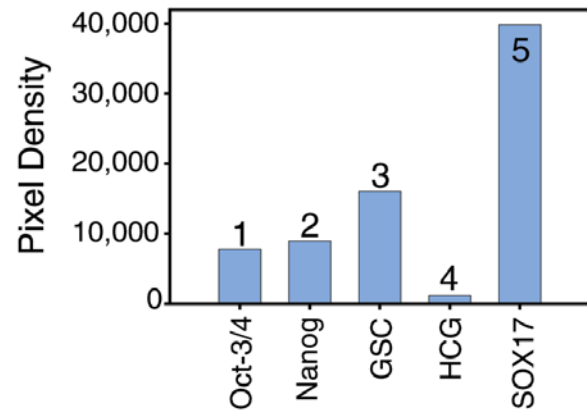
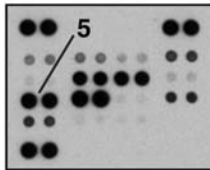
A Undifferentiated



B Mesendoderm



C Endoderm



D Trophectoderm

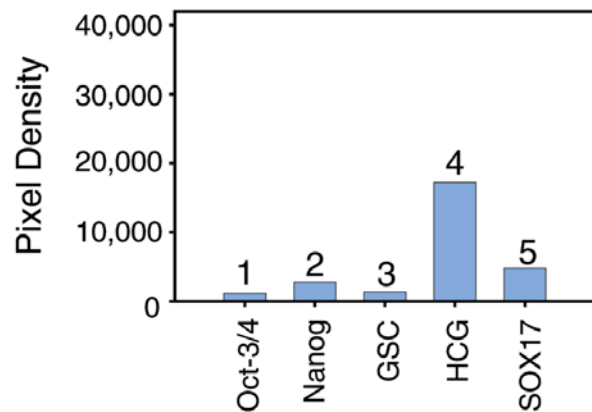
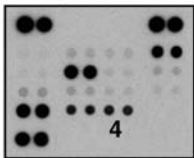
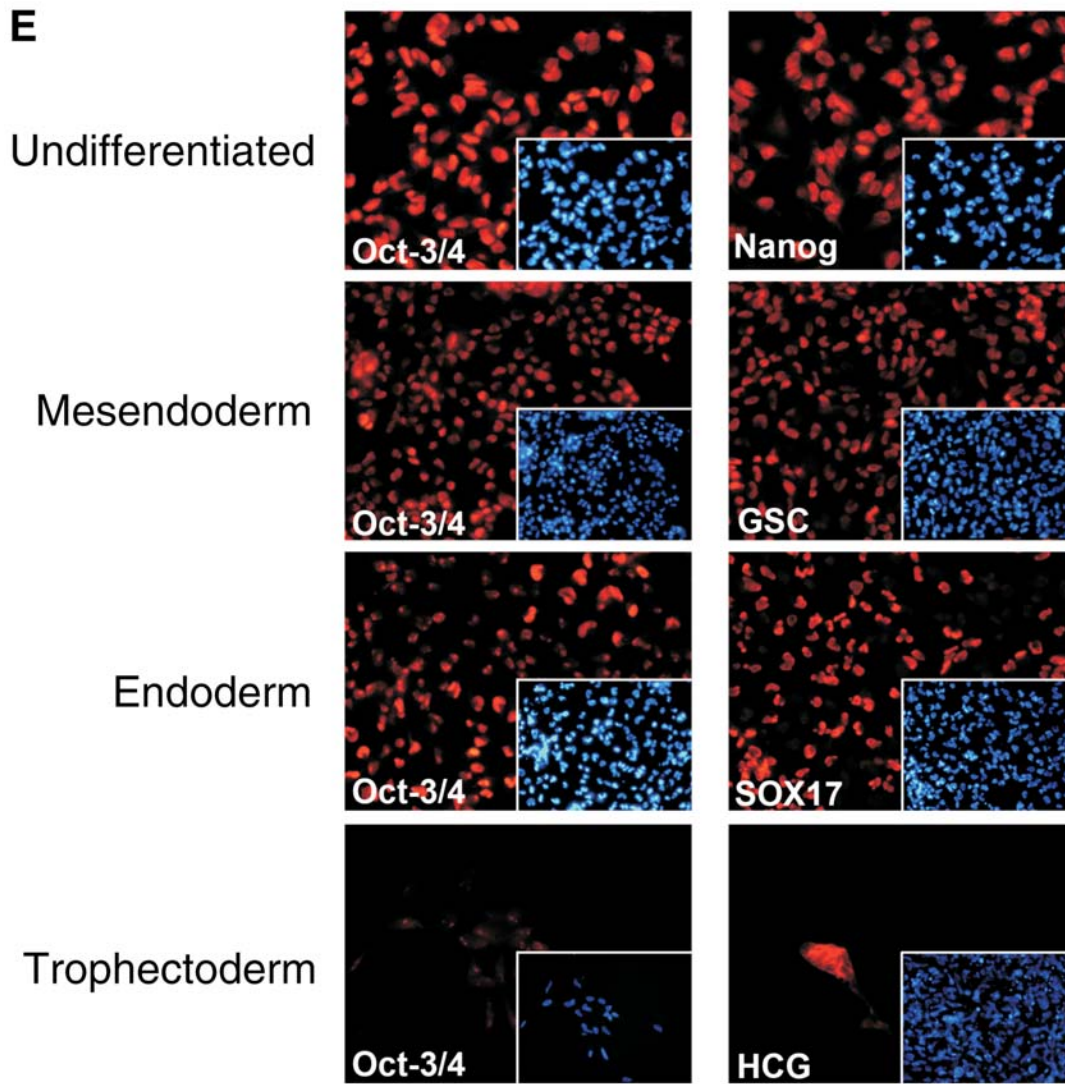


Figure 1A - D: The Human Pluripotent Stem Cell Array detects multiple stem cell markers in differentiated BG01V cell extracts. Arrays were incubated with 200 μ g of each cell extract shown above. Array images shown were collected from 3 minute exposures to X-ray film.



For full color images, please refer to our website
www.RnDSystems.com/pdf/ARY010.pdf.

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 subsequently grown in media containing only recombinant human/mouse/rat Activin A for two days. For trophoderm 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

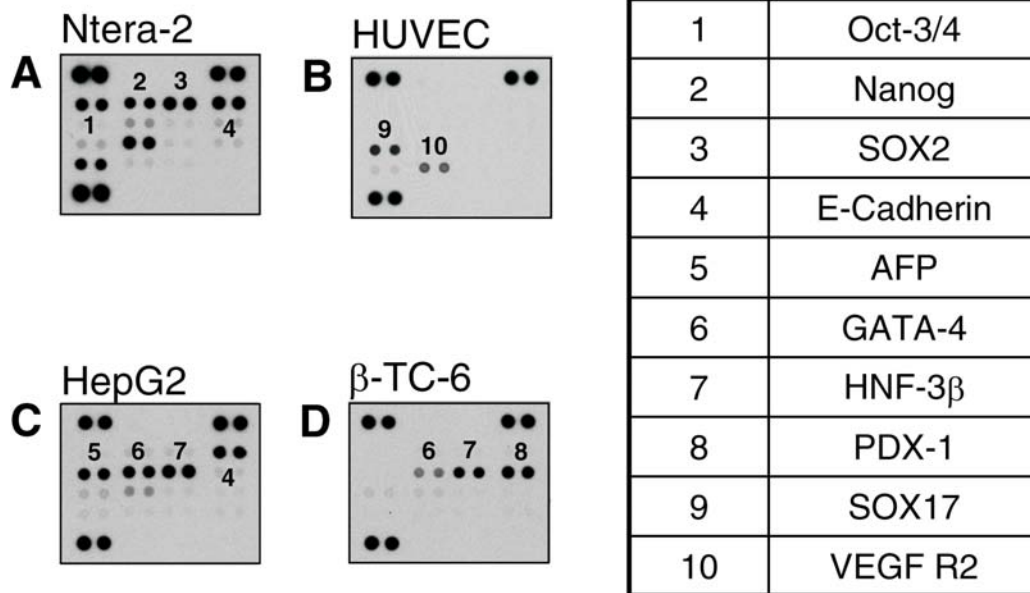


Figure 2: The Human Pluripotent Stem Cell Array detects multiple protein markers in various cell extracts. Arrays were incubated with 200 μ g of each cell extract shown above. Array images were collected from 3 minute exposures to X-ray film.

SENSITIVITY

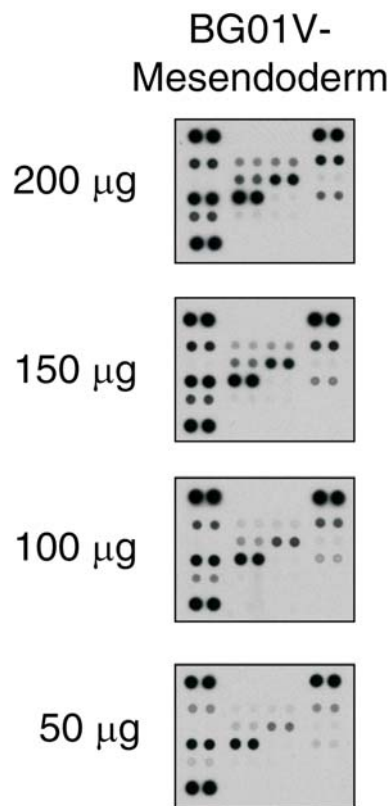


Figure 3: Signal intensities for stem cell markers may be modulated by the quantity of cell extract incubated with the Human Pluripotent Stem Cell Array. Arrays were incubated with 50 - 200 µg of BG01V mesendoderm differentiated extracts as shown above. Array images were collected from 2 minute exposures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

APPENDIX

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

Coordinate	Target/Control
A1, A2	Positive Control
A7, A8	Positive Control
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-3 β /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	Positive Control

BG01V cells are licensed from Novocell, Inc.