Profiling Human Pluripotent Stem Cell Markers Using Multiplexed Antibody Arrays

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ABSTRACT

The Human Pluripotent Stem Cell Antibody Array (Catalog # ARY010) is a rapid and economical tool designed to simultaneously detect the relative levels of 15 different stem cell markers in a single sample. Capture antibodies have been carefully selected for each analyte and spotted in duplicate on nitrocellulose membranes. Cellular extracts are diluted and incubated with the antibody 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. Analysis of undifferentiated and differentiated BG01V cell extracts show changes in stem cell marker levels throughout the differentiation process. The results obtained with the Antibody Array were confirmed by immunocytochemistry for each stage of BG01V differentiation.

Oct-3/4 and GATA-4 expression were also measured using Western blot and RT-PCR for undifferentiated and mesendoderm differentiated BG01V cell extracts. Array experiments can be completed with 5.5 hours of hands-on time and do not require the use of specialized equipment. Thus, the Human Pluripotent Stem Cell Antibody Array offers a sensitive and efficient means to detect changes in multiple protein markers of differentiation that complements other available tools such as PCR and microscopy.

METHODS

1. Block arrays for 1 hour at room temperature.
2. Incubate arrays with diluted cellular extracts overnight at 2-8 ˚C.
   > Wash arrays 3 x 10 minutes at room temperature.
3. Incubate arrays with a cocktail of biotinylated detection antibodies for 2 hours at room temperature.
   > Wash arrays 3 x 10 minutes at room temperature.
4. Incubate arrays with streptavidin-HRP solution for 30 minutes at room temperature.
   > Wash arrays 3 x 10 minutes at room temperature.
5. Incubate arrays with chemiluminescent reagents.
   > Collect multiple exposures.

Figure 1.

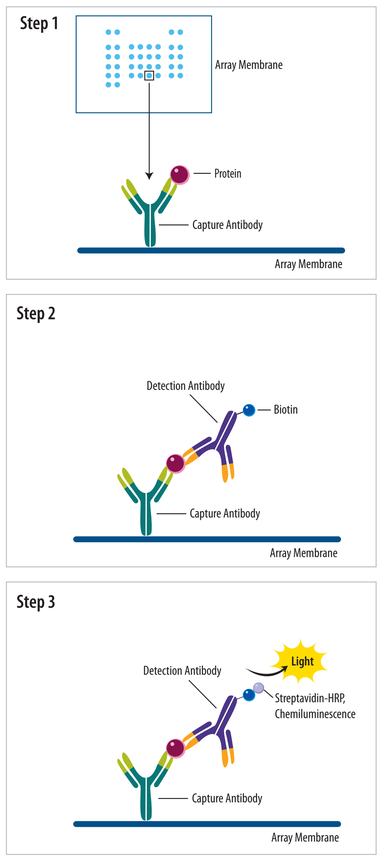


FIGURE 1: R&D Systems Proteome Profiler Antibody Array Detects Multiple Proteins in a Single Sample. Proteome Profiler Antibody Arrays are designed using carefully selected capture antibodies that are spotted in duplicate on nitrocellulose membranes. When these membranes are incubated with experimental samples, capture antibodies printed on the membranes bind to their specific target proteins **(Step 1)**. Captured proteins are detected with carefully selected detection antibodies conjugated with biotin **(Step 2)**. Proteins are visualized using chemiluminescent detection reagents, which produce a signal that is proportional to the amount of analyte bound **(Step 3)**.

Figure 2.

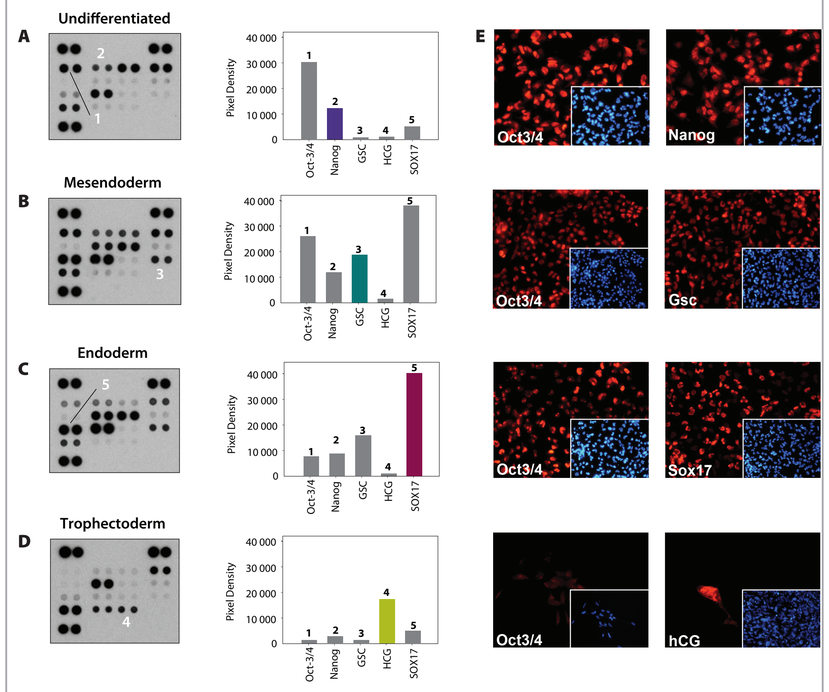


FIGURE 2: Profiling Stem Cell Markers in BG01V Extracts. (A-D) The Human Pluripotent Stem Cell Array detects multiple stem cell markers in differentiated BG01V cell extracts. Arrays were incubated with 200 µg of each cell extract shown above. Array images were visualized using chemiluminescent reagents and 3 minute film exposure. **(E)** Cells were stained with anti-human Oct-3/4 (Catalog # AF1759), anti-human Nanog (Catalog # AF1997), anti-human SOX17 (Catalog # AF1924), anti-human Goosecoid (Catalog #AF4086), or anti-human HCG (Catalog # MAB4169). DAPI nuclear staining is shown in each image inset. Extracts were prepared from BG01V hES cells grown under undifferentiated conditions in MEF Conditioned Media (R&D Systems, Catalog # AR005) supplemented with rhFGF basic (Catalog # 4114-TC). For mesendoderm differentiation, cells were grown in serum-free media in the presence of rmWnt-3a (Catalog # 1324-WN) and rh/m/rActivin A (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 rh/m/rActivin A for two days. For trophectoderm differentiation, cells were grown in MEF Conditioned Media supplemented with rhFGF basic and rhBMP-4 (Catalog # 314-BP) for seven days.

Figure 3.

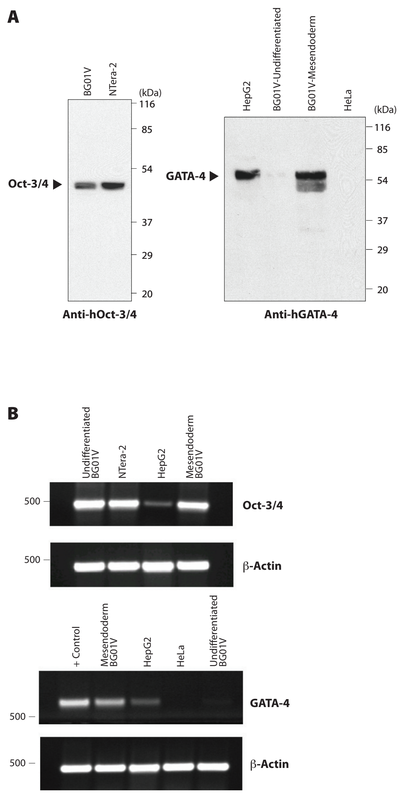
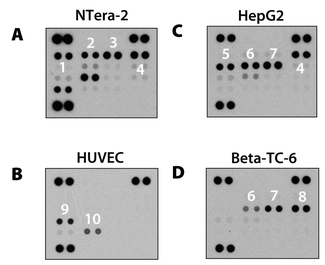


FIGURE 3: Stem Cell Array Data was Confirmed by Western Blotting and RT-PCR. (A) Oct-3/4: Extracts from undifferentiated BG01V and NTera-2 cells were resolved by SDS-PAGE and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL anti-human Oct-3/4 (R&D Systems, Catalog # MAB1759). GATA-4: Extracts from HepG2, undifferentiated BG01V, mesendoderm differentiated BG01V, and HeLa cells were resolved by SDS-PAGE and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL anti-human GATA-4. **(B)** Oct-3/4: RT-PCR analysis of human Oct-3/4 in undifferentiated BG0IV, NTera-2, HepG2, and mesendoderm differentiated BG01V cells. Oct-3/4 primers amplify a 486bp fragment from cDNA (and pseudogene) and a 1031bp fragment from genomic DNA. Gata-4: RT-PCR analysis of hGATA-4 in pooled cDNA positive control, mesendoderm differentiated BG01V, HepG2, HeLa, and undifferentiated BG01V cells. GATA-4 primers amplify a 569 bp fragment from cDNA and a 9413 bp fragment from genomic DNA.

Figure 4.



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

FIGURE 4: The Human Pluripotent Stem Cell Array Detects Multiple Protein Markers in Various Cell Extracts. Arrays were incubated with 200 µg of each cellular extract shown above. Array images were visualized using chemiluminescent reagents and 3 minute film exposure.

Figure 5.

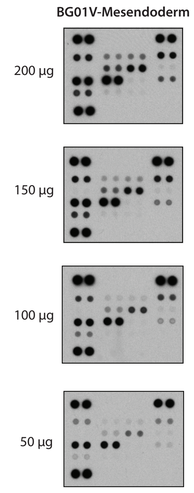


FIGURE 5: Signal Intensities for Stem Cell Markers May be Modulated by the Quantity of Cell Extract Incubated with the Human Pluripotent Stem Cell Array. Arrays were incubated with 50-200 µg of mesendoderm-differentiated BG01V extracts. Array images were visualized using chemiluminescent reagents and 2 minute film exposure.

Table 1.

Human Pluripotent Stem Cell Array Analytes		
Oct-3/4	GATA-4	TP63/TP73L
Nanog	HNF-3β/FoxA2	Goosecoid (GSC)
SOX2	PDX-1/IPF1	Snail
E-Cadherin	SOX17	VEGF R2 /KDR/Flk-1
α-Fetoprotein (AFP)	Otx2	HCG

SUMMARY

Analyzing the expression profiles of stem cell markers is essential for understanding the roles these transcription factors and signaling molecules play in mechanisms required to sustain pluripotency and direct cell differentiation. The Human Pluripotent Stem Cell Array (Catalog # ARY010) is an effective tool for screening 15 stem cell markers in a single sample without performing numerous immunocytochemistry or Western blot experiments.

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