ECM Select® Array Kit Ultra - 36

EXTRACELLULAR MATRIX SCREEING ARRAY
Catalog Number 5170

Product Description

ECM Select® Array Kit Ultra-36, an extracellular matrix screening array, contains a microscope glass slide functionalized with a proprietary hydrogel on the surface. Thirty six (36) extracellular matrix (ECM) conditions are deposited onto the hydrogel surface as printed array spots – all ECM conditions are derived from human sources. Each spot has diameter of 400 um and is printed in replicates of 9 (Figure 1). ECM's are localized within each spot without cross diffusion from neighboring spots, therefore each spot represents an independent 'well' or experiment.

Mammalian cells exist in unique micro-environments *in vivo* that affect their behavior (proliferation, differentiation, death, etc.) and function. When cells are cultured *in vitro*, it is necessary to recapitulate such microenvironment for physiologically relevant growth. Among many factors affecting cell behavior *in vivo*, ECMs play a significant role in determining cellular functions. Currently, finding the optimal ECM for cell culture is a trial and error process requiring large numbers of cells as well as a significant time and cost commitment. The *ECM Select® Array Kit Ultra-36* permits 36 ECM's and ECM combinations to be screened in parallel with a small number of cells in a short time period.

Cells of interests are seeded onto the slide in a 4-chambered culturing plate (included) and allowed to be cultured in the incubator for desired amount of time. Cell morphology, attachment and growth can be visualized using a bright field microscope. Specific cellular behaviors can be monitored by staining the cells on the slide using specific fluorescence based marker. Fluorescence signal can be detected using fluorescence microscope imaging system.

The *ECM Select® Array Kit Ultra-36* is simple to use in comparison to performing the same experiment in multi-well tissue culture plates. Cell seeding, fixing, washing and process step are done in one single solution exchange, eliminating multi-well solution handlings. From start to finish, the optimal ECM for culturing cells can be determined in less than 24 hours for most cell types.

Precautions and Disclaimer

This product is for R&D use only and is not intended for human or other uses. Please consult the Material. Safety Data Sheet for information regarding hazards and safe handling practices.

Storage, Shelf Life and Stability

The shelf life of ECM Select® Ultra Array Kit is a minimum >12 months when stored at -10 to -30°C. Shelf life testing is on-going. Repeat freezing and thawing is not recommended but limited freezing and thawing does not seem to have negative effects on the product. Storing at colder temperatures is not recommended.

From our stability studies, ECM Select® Ultra Array Kit performs adequately when stored at 2 to 8°C for up to 3-4 days. However, after 2 weeks at 2 to 8°C, some of the extracellular matrix conditions are degraded in their cell attachment ability. After 1 month at 2 to 8°C, almost all the ECM conditions on the array slide lose their cell attachment ability.

Protocol

Seeding cells onto the slide

1. Prepare 250,000 desired cells in 5 ml complete culture growth media (50,000 cells/ml).

2. Remove *ECM Select® Array Kit Ultra-36* from -20ºC freezer and put it under a sterile laminar flow hood. Open the foil pouch and remove contents from the foil pouch. First, take out the cell culture plate from the plastic bag and remove the lid. Then open cap on the slide holder and gently remove the slide from the slide holder. Put the slide in one of the chambers of the cell culture plate. Take precaution while removing the slide, using sterile technique such as wearing sterile gloves or using a sterile forceps and only touch the edge of the slide. Make sure the printed spot side is up (numbers and letter on slide should be legible).

3. Add 5 ml of sterile phosphate buffered saline (PBS) onto the slide. Make sure that the whole slide is immersed in the PBS solution and avoid trapping air bubble underneath of the slide. Wash the slide by rocking it gently back and forth several times and then aspirate the PBS solution away.

   Do not touch the surface of the slide.

4. Add 5 ml of the desired culture media (same media for culturing cells) and make sure the whole slide is immersed in the media. Wash the slide by rock it gently back and forth several time and then aspirate the media solution away.

   Do not touch the surface of the slide.

5. Mix the prepared 250,000 cells briefly by gently pipetting up and down and immediately add the cells directly onto the slide. Evenly distribute the cells by moving the pipette back and forth while dispensing the cells onto the slide.

   Do not touch the surface of the slide. Replace the lid onto the plate.

6. Carefully transfer the 4-well plate containing the slide to the incubator and culture the cells at 37ºC, 5% CO_2 overnight. (minimum 12 hours to allow efficient cell attachment up to a maximum of 3 days).

Note: Array spots may be visible on the slide surface when removed from the slide holder. Upon washing with PBS/Media, these spots may disappear. This is normal and will not affect ECM array performance.

Note: For cell lines, 12 hours should be sufficient to see cell attachment. For cells freshly isolated from the tissue or cells previous frozen, it may require up to 2 days before seeing significant cell attachment.

Note: If cell proliferation study is desired, reduce the cell number to allow extra space on the spot for cell to divide. It is recommended to start with 100,000-150,000 cells dependent on the size and morphology of the cells. The larger size of cells, the smaller number of cells is needed.

Washing and Observing cells on the slide

1. After the cells have been incubated for at least 12 hours, remove the plate from the incubator and place the plate under the phase contrast microscope for observation, starting at 5X objective lens. Gently move the plate back and forth to distinguish the non-adherent floating cells from the attached cells.

Note: Because of the non-fouling nature of the hydrogel surface on *ECM Select*® slide, cells will preferentially attach to the spots where appropriate extra cellular matrices are printed. Cells are confined within the spots.

2. Remove the non-adherent floating cells by aspirating the media away; making sure the slide surface is not disturbed. Wash the slide twice with 5 ml culture media each, and then leave the slide in 5 ml of culture media.

3. Observe the cell attachment under the phase contrast microscope for each block of spots. Each block represents a unique ECM with 9 replicates of spots. For ECM identity of each block, see Figure 2.

4. Cells can be fixed at this stage or can be cultured for additional 3 days on the slides.

Fixing cells on the slide

1. Remove the culture media by aspirating the media away. Wash the cells on the slide twice with cold 5 ml 1X Hank's Balanced Saline solution (HBSS) with Ca^{2+} and Mg^{2+}.

2. Fix the cells on the slide by adding 5 ml cold 4% paraformaldehyde (PFA) prepared in 1x PBS solution. Leave the slide in the PFA solution at 4ºC for 5 minutes, then at room temperature for additional 10 minutes.

3. Remove PFA solution and then wash the slide twice with 5 ml 1x HBSS solution each. Slide can be stored in 1x HBSS solution at 4ºC for future staining or can be processed immediately afterward.

Nuclear- and Immuno- staining cells on the slide

1. Cells on the slide can be stained using many available nuclear staining reagents, such DAPI, PoPo-3, DraQ, etc. Following the manufacturers' instructions.

2. For specific antibody staining, following the manufacturer's instructions for immunocytochemistry dilution recommendations.

3. After finishing staining, wash the slide twice with 5 ml cell culture H_2O at 5 minutes each. Then remove the slide from the plate and dry the slide on the rack in the dark.

4. Slide can be loaded onto a fluorescence microscope directly for observation.

Note: Do NOT use any mounting media, such as Vectashield.

Note: Do NOT use inverted position for observation commonly used for oil lenses.

Applications

The *ECM Select® Array Kit Ultra-36* has been successfully used for cell lines, such as MCF-7, CHO, HUVEC, fibroblast, MG-63, Jurkat cells, as well as primary cells, such as mouse Cardiac Progenitor cells, rat neo-natal cardiomyocyte, human oligodendrocyte precursor cells using this protocol.

Reference Extra Cellular Matrix Products

ECM Component	Advanced BioMatrix Catalog Number
Collagen, Human, Type I	5007
Collagen, Human, Type III	5021
Collagen, Human, Type IV	5022
Collagen, Human, Type V	Not Available (1)
Laminin, Human	Not Available
Fibronectin, Human	5050
Vitronectin, Human	5051
Tropoelastin, Human, Recombinant	5052

(1) Type V Human Collagen is not available. However, Type V Bovine Collagen (Catalog 5168-0.5MG) is available from Advanced BioMatrix

References

Brafman D., Shah KD, Fellner T, Chien S, Willert K. (2009) "Defining Long-Term Maintenance Conditions of Human Embryonic Stem Cells With Arrayed Cellular Microenvironment Technology." Stem Cells Dev., 18(8): 1141-1154.

Brafman, D, de Minicis S, Seki E, Shah KD, Teng D, Brenner D, Willert K, Chien S. (2009) "Investigating the role of the extracellular environment in modulating hepatic stellate cell biology with arrayed combinatorial microenvironments". Integr. Biol., 1(8): 513 – 524.

Figure 1 *ECM Select®* Array Slide design.
Each square contains a unique ECM condition with 9 replicating spots.

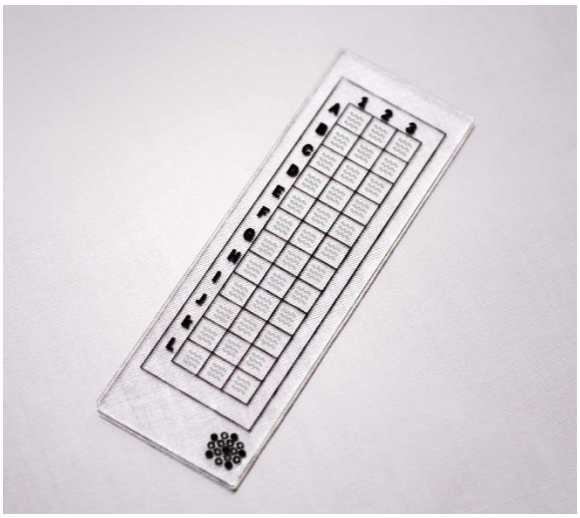

Figure 2 *ECM Select®* Array Slide components identification.

	1	2	3
A	Collagen I (250µg/ml)	Collagen I (125µg/ml) Collagen III (125µg/ml)	Fibronectin (125µg/ml) Collagen IV (125µg/ml)
B	Collagen III (250µg/ml)	Collagen I (125µg/ml) Collagen IV (125µg/ml)	Fibronectin (125µg/ml) Collagen VI (125µg/ml)
C	Collagen IV (250µg/ml)	Collagen I (125µg/ml) Collagen V (125µg/ml)	Fibronectin (125µg/ml) Laminin (125µg/ml)
D	Collagen V (250µg/ml)	Collagen I (125µg/ml) Collagen VI (125µg/ml)	Fibronectin (125µg/ml) Vitronectin(125µg/ml)
E	Collagen VI (250µg/ml)	Collagen I (125µg/ml) Fibronectin (125µg/ml)	Laminin (125µg/ml) Collagen IV (125µg/ml)
F	Fibronectin (250µg/ml)	Collagen I (125µg/ml) Laminin (125µg/ml)	Laminin (125µg/ml) Collagen VI (125µg/ml)
G	Laminin (250µg/ml)	Collagen I (125µg/ml) Vitronectin (125µg/ml)	Vitronectin (125µg/ml) Collagen IV (125µg/ml)
H	Vitronectin (250µg/ml)	Collagen I (125µg/ml) Topoelastin (125µg/ml)	Vitronectin (125µg/ml) Collagen VI (125µg/ml)
I	Tropoelastin (250µg/ml)	Fibronectin (83.3µg/ml) Laminin (83.3µg/ml) Collagen I (83.3µg/ml)	Vitronectin (125µg/ml) Laminin (125µg/ml)
J	Collagen IV (125µg/ml) Collagen VI (125µg/ml)	Fibronectin (83.3µg/ml) Laminin (83.3µg/ml) Collagen IV (83.3µg/ml)	Vitronectin (125µg/ml) Topoelastin (125µg/ml)
K	Collagen III (125µg/ml) Collagen V (125µg/ml)	Vitronectin (62.5µg/ml) Laminin (62.5µg/ml) Collagen I (62.5µg/ml) Collagen IV (62.5µg/ml)	Topoelastin (125µg/ml) Collagen IV (125µg/ml)
L	**Negative Control (250 µg/ml)**	Fibronectin (62.5µg/ml) Laminin (62.5µg/ml) Collagen I (62.5µg/ml) Collagen IV (62.5µg/ml)	Topoelastin (125µg/ml) Collagen VI (125µg/ml)

