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TECHNICAL BULLETIN

Plaque Assay-Ready 12 Well Pre-Plated A549 Cells

Catalog Number: CLX-PLQ-12-549

Storage Temperature: -80 °C

Product description

Pre-plated assay ready A549 cells are Human Caucasian lung carcinoma cells that have been frozen adhered to 12 well tissue culture plates with the use of Cryologyx's patented CryoShield™ formulation. The plates provided have been specifically designed for use in viral titering plaque assays. Simply thaw the cells and use them 24 hours post-thaw. Near total cell recovery is expected post-thaw, with cells retaining healthy morphology, immediate proliferative capacity, metabolic activity and membrane integrity.[1] In addition, no cell debris is expected.

The simplicity of Plaque Assay-Ready 12 Well Pre-Plated A549 Cells removes the need for cell handling, excessive plastic waste and risks of phenotypic drift from cell passaging, whilst facilitating high throughput screening and maximising the time spent conducting 'value adding' activities.

Components

1 x Plaque Assay-Ready 12 Well Pre-Plated A549 Cells Catalog No. CLX-PLQ-24-549

Reagents and Equipment Required but Not Provided

- Ham's F-12K (Kaighn's) Medium
- Fetal Bovine Serum Albumin
- Antibiotic-Antimycotic (optional)

Storage/Stability

Plaque Assay-Ready 12 Well Pre-Plated A549 Cells is shipped in dry ice storage and must be transferred into a -80 °C freezer as soon as possible. Once thawed, cells should be stored in an incubator set at 37 °C and 5% CO_2. Use plates within 3 weeks for optimal results.

Procedure

Thawing Cryopreserved Pre-Plated Cells

1. Prepare complete Ham's F-12K (Kaighn's) Medium by supplementing Ham's F-12K (Kaighn's) Medium with 10% fetal bovine serum albumin and 1% antibiotic-antimyotic (optional)
2. Remove no more than 2 x 12 well plates from the -80 °C freezer at a time

3. Thaw cells by adding 1 mL of warm complete cell culture media (warmed to 37 °C) to each well.
4. Place the plates in an incubator set at 37 °C and 5% CO_2 for 24 hours.

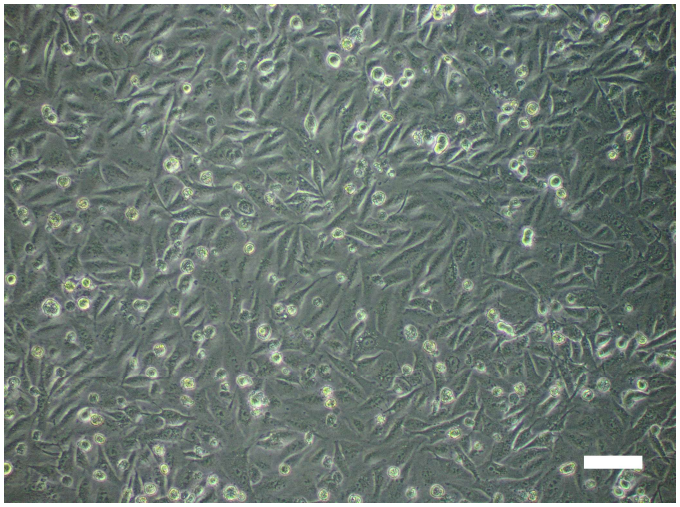

Figure 1. Sample phase contrast image of A549 cells from a Plaque Assay-Ready plate 24 h following thawing.

Plaque Assay

1. The thawed Plaque Assay-Ready Pre-Plated A549 Cells should already be at the optimum confluency (>80%). If slightly lower density is present, wait a further 24 hours to reach the desired confluency level.
2. Remove the growth medium and wash cells with Dulbecco's Phosphate-Buffered Saline (DPBS) three times to ensure no residual FBS remains.
3. Add diluted virus, in an appropriate medium, to each well using multiple wells per dilution (10^{-1} – 10^{-11} serial dilutions with two technical repeats) and leave 2 wells as a negative control. Use a sufficient volume of inoculum to cover the cells, whilst keeping the volume as low as possible to maximize viral contact with the monolayer. For a 12 well plate, 200 μL should be sufficient.
4. Gently rock the plates every 20 min to ensure even coverage and prevent the cellular monolayer from drying.
5. Incubate dishes for 1 – 2 hours to allow viral adsorption under the appropriate temperature and atmospheric conditions for the recommended incubation period.
6. Remove the inoculum and wash with basal medium.
7. Overlay the cells with overlay medium and incubate cell monolayers until the presence of foci or plaques are observed (check daily). If applicable, remove overlay and carry on with fixation and staining.

   Suitable overlay medium include a 1:1 mixing of warm 2x Eagle's minimum essential medium (EMEM), or another plaque medium, with 1.2 – 2.4% Avicel. For an agarose overlay, use a 1:1 mixture of warm 2x EMEM (or another plaque medium) and a stock of heated 0.6% agarose (56 °C for 30 mins in a water bath) and apply to cell monolayers. Agar will begin to solidify below 42 °C.

8. Observe cell monolayers daily for the presence of foci or plaques and determine viral titer by counting well isolated plaques (select a serial dilution with this) and use the following formula to determine the titer (pfu/mL) of the viral stock:

$$\frac{Pfu}{mL} = \frac{Avg.\#\ of\ plaques}{Dilution\ factor\ \mathrm{x}\ volume\ of\ diluted\ virus\ added\ (mL)}$$

References

https://pubs.acs.org/doi/full/10.1021/acs.biomac.2c00791