CRYOLOGYX

Simplifying and accelerating cell-based assays

ToxReady™ A549 96 Well Plates

General thaw-and-use protocol to prepare ToxReady™ A549 96 well plates for any assay.

REF: A5490096PSCLLOW, A5490096PSCLMID, A5490096PSCLHIG

Introduction

ToxReady™ A549 96 well plates remove the need for routine cell culture by offering A549 cells cryopreserved adhered onto a 96 well plate. Simply store the well plates in a -80 °C freezer until they're required for use.

This protocol outlines the simple steps necessary to ensure the optimal revival of A549 cells in ToxReady™ A549 96 well plates, making them assay-ready 24 hours post-thaw. Once thawed, ToxReady™ A549 96 well plates can be used for any subsequent application. Application notes are available for specific assay protocols.

ToxReady™ A549 96 well plates are provided in three confluency levels, low (20 – 30%), medium (40 – 60 %) and high (>70%) to ensure compatibility with all major assays. Select the confluency level based on the desired protocol.

Storage

Store in a -80 °C freezer. Use by expiry date.

Not Included

Component	Quantity	Description	Storage
Ham's F-12K (Kaighn's) Medium	500 mL	Base medium (Example: Thermo Fisher 21127022)	4°C
Fetal Bovine Serum	50 mL	Growth supplement (e.g., Merck F7524)	-20°C
Antibiotic-Antimycotic (100x) (optional)	5 mL	Contamination prevention (e.g., Thermo Fisher 15240062)	-20°C

Prepare in Advance

- Complete Ham's F-12K (Kaighn's) Medium:

1. Remove 55 mL of Ham's F-12K (Kaighn's) Medium
2. Add 50 mL of fetal bovine serum (final concentration 10%)
3. (optional) Add 5 mL of antibiotic-antimyotic (final concentration 1%)

- Pre-warm medium to 37 °C before removing plates from the -80 °C freezer.

Revision 2 Updated 24-08-2023

General Protocol

ToxReady™ Plates

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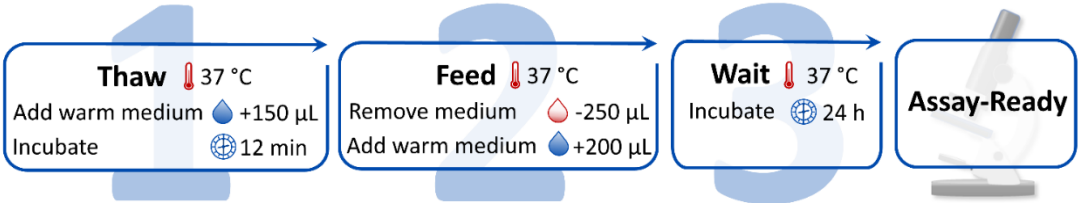
Procedure



Method

1.1 Remove no more than 3 x ToxReady™ A549 96 well plates from the -80 °C freezer.

1.2 Remove protective film and add 150 µL of warm complete cell culture media (warmed to 37 °C) to every well.

1.3 Place the plates in an incubator set at 37 °C and 5% CO_2 for 12 mins to allow the cells to thaw. Do not stack the plates.

1.4 Remove the plate from the incubator and ensure that the cells have completely thawed.

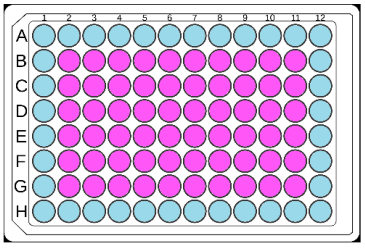
Note: To confirm this, no ice should be present at the bottom of the wells. If there is, return to the incubator until completely thawed.

2.1 Remove the medium + cryoprotectant solution (250 µL)

2.2 Add 200 µL of warm complete cell culture media (warmed to 37 °C).

3.1 Place the plates in an incubator set at 37 °C and 5% CO_2 for 24 hours.

Cells are ready for use in all major assays. No cells are present in rows A and H and columns 1 and 12 (highlighted blue) to avoid assay edge effects.



Notes

For additional product information please consult the product specification document an certificate of analysis. FAQs and Assay Ready protocols can be found at www.cryologyx.com under the resources section.

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