ARCTis™ Oncology
Quick Start Guide

Thank you for choosing InSphero's ARCTis Oncology Cryo-Tissues for your 3D cell culture experiments. This Quick Start Guide contains important information to get you started immediately. For detailed instructions please refer to the Product Manual and additional resources on shop.insphero.com.

ARCTis™ Oncology Platform

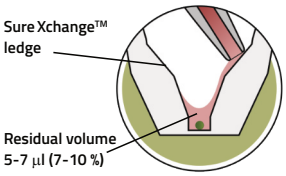
A. Akura™ 96 Spheroid Plate
B. Cryopreserved Cell Suspension
C. Reaggregation Medium
D. Tilting Stand
E. Tumor Maintenance Medium

Thawing ARCTis™ Oncology Plates

The total work effort is roughly 30 min. Preparing multiple plates can be done in parallel depending on available automation infrastructure and sufficient number of Tilting Stands (D). Each plate needs to thaw on its own Tilting Stand.

Use prewarmed InSphero's Reaggregation Medium (C) at 37 °C for all pipetting steps. Prewarm the Tilting Stand (D) by placing it in the incubator and let it warm up to 37 °C (10-15 min) while preparing the cell culture hood.

1. Remove ARCTis™ plate from -80 °C freezer and transfer to cell culture lab on dry ice.
2. Open the packaging bag on the triangular side carefully and remove the plate.
3. Immediately place it on the prewarmed Tilting Stand inside the incubator (37 °C, 5 % CO_2) and let the plate thaw for 7 min.
4. Transfer the ACTis™ plate to cell culture hood and carefully remove the sealing foil.
5. Gently and stepwise dispense Reaggregation Medium (C) to each well according to the following procedure:
   i. Gently dispense20 µl of medium - incubate for 1 min
   ii. Gently dispense 20 µl of medium - incubate for 1 min
   iii. Gently dispense 60 µl of medium - incubate for 1 min
   iv. Gently dispense 80 µl of medium - incubate for 1 min
   → Total volume per well now 200 µl



6. Centrifuge plate at 250 rcf for 2 min
7. Slowly and carefully aspirate supernatant (10-20 µl/s if using an automated pipette) with the pipette tip in contact with the SureXchange™ ledge (see illustration), leaving a residual volume of 5 - 7 µl in each well
8. Dispense gently 70 µl medium (80-90 µl/s) in each well
9. Centrifuge the ARCTis™ plate at 250 rcf for 2 min
10. Transfer ARCTis™ plate to incubator and place on Tilting Stand for at least 48 - 72 h

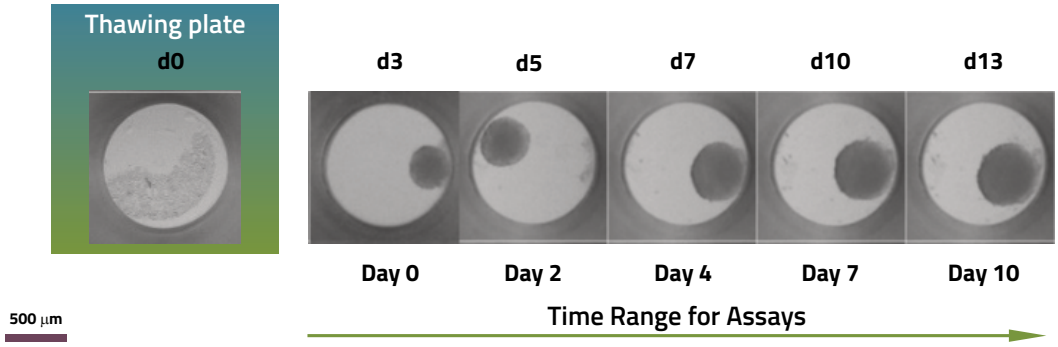
Note: Depending on cell line your plate is assay-ready in 3-5 days. Please refer to the recommended recovery time illustrated on the specifications sheet given for each cell line. Your spheroids should appear similar to the ones shown in the illustration on next page.

Medium Exchange/Dosing

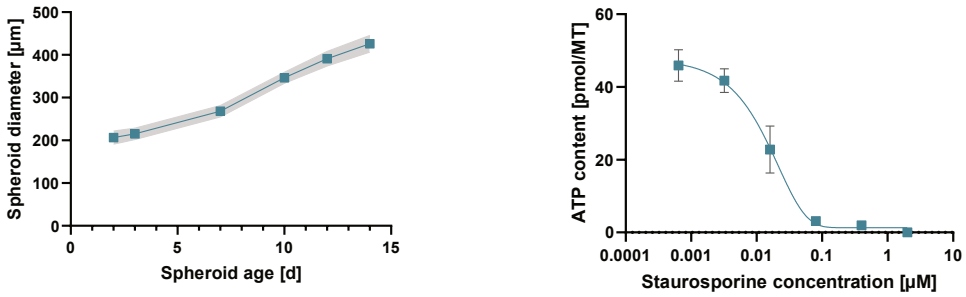
Set pipette to 75 µl aspiration, place tip on ledge to remove slowly all medium above the ledge. Replace with 70 µl fresh medium well using a 12-channel pipette with a speed of 10-20 µl/s. Have your compound diluted in the Maintenance Medium according to your test scheme.

Technical Note

Each ARCTis™ Oncology plate contains 20 µl cryopreserved cell suspension of 500 to 2000 cells per well. The number of cells has been optimized depending on the observed doubling time of the respected cell line. As a result, the formed spheroid has the correct size to allow for a testing period of 10 days after reaggregation.
Throughout this 10-days test-window, a variety of different endpoints can be measured: such as growth rate, ATP content, viability or GFP levels if applicable.



Brightfield image series of an untreated HCT116 spheroid illustrating a typical growth over 13 days from thawing (d0) to last day of measurement (Day 10).



Left) typical growth curve for HCT116 spheroids over 13 days; **Right)** Dose response for HCT116 spheroid using Staurosporin

For more details please refer to the ARCTis™ Product Manual available on our website.

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InSphero is ISO 9001:2015 certified

Flyer Version: 2023-Jul Vers 1