SARTORIUS

Product Protocol

Adherent Cell Lifting Protocol

Background

This protocol describes a simple workflow for lifting adherent cells and transferring them onto the iQue® 3 for analysis. This protocol is applicable but not limited to proliferation, cell health and immune cell killing assays.

The lifting protocol has been optimized to minimize sample manipulation during cell transfer. This method can also be used to combine the Incucyte® and iQue® 3 instruments for analysis of cells on both platforms.

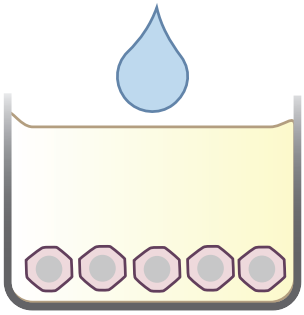
Required materials

Cells of interest (adherent)
Cell culture media
96-well flat-bottom microplate
(e.g. Corning Cat. No. 3595)
96-well V-bottom microplate (e.g. Costar Cat. No. 3363)
Accutase (e.g. Gibco Cat.No. A1110501)
PBS
PBS + 2% FBS

General guidelines

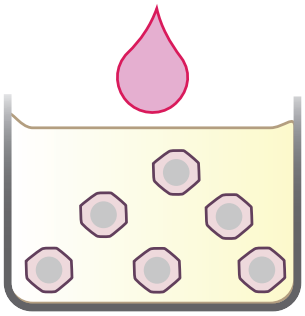
This lifting protocol can be applied to a multitude of adherent assays. Therefore, set up the adherent cell assay plate according to the particular protocol, leave the assay for the pre-determined time and then follow the steps below to allow analysis on the iQue® 3.

1. Wash with PBS



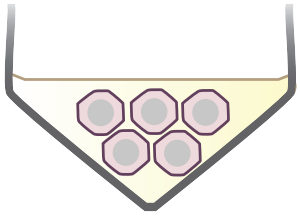
Centrifuge (300 xg, 5 mins). Remove media with a multichannel pipette (leave 50 µL in wells). Wash 1x with PBS (100 µL/well).

2. Add Accutase to lift cells



To lift cells from the plate, add Accutase (50 µL/well) for 10 minutes at 37°C then shake for 2 mins (1400 rpm).

3. Transfer to V-bottom plate



Transfer cells to a V-bottom plate for endpoint analysis with the iQue®.

Adherent cell protocol:

1. Prepare cell sample:

a. Centrifuge plate at 300 xg for 5 minutes and remove supernatant gently using a multichannel pipette, leaving at least 50 µL media in the wells.

Note: If cytokine quantification required, take 10 µL supernatant samples from wells prior to using this protocol. Follow cytokine detection steps within the appropriate iQue® kit.

b. Wash once with 100 µL/well PBS and centrifuge at 300 xg for 5 minutes to ensure all cells remain in the wells.

c. Gently remove 100 µL/well PBS and add 50 µL/well Accutase for 10 minutes at 37 °C followed by a 2 minute shake at 1400 rpm.

d. Add 100 µL media to quench, triturate the sample and then transfer to a V-bottom plate.

Note: Use a microscope to confirm that the cells have been lifted and transferred from the assay plate, if cells remain repeat steps b & c.

e. Centrifuge the plate at 300 xg for 5 minutes, aspirate supernatant and re-suspend in 20 µL of PBS (+ 2% FBS).

f. Add appropriate reagents as per iQue kit protocols.

2. Plate Acquisition and Data Analysis

a. Launch iQue Forecyt® Software

b. In the Design section, assign wells to sample adding any necessary standards to ensure proper plate layout.

c. In the Protocol section: Adjust sip times if desired.

d. Click "Run" on the Controller to acquire the plate.

Table 1: List of cell types detailing suitability for use with lifting protocol.

Type	Cell Line	Compatible
Breast	AU565	✓
	BT474	✓
	HCC38	✓
	MCF-7	✓
	MDA-MB-231	✓
	SK-Br-3	✓
	T-47D	✓
Ovarian	SKOV-3	✓
Lung	A549	✓
Colon	HT1080	✓
Prostate	PC-3	✓
Macrophage (mouse)	J774A.1	✓*
	RAW264.7	✗

* Compatible but with further optimization steps required

Sales and Service Contacts

For further contacts, visit
www.sartorius.com

Essen BioScience, A Sartorius Company

www.sartorius.com/ique

Publication No. #17109 Rev A.

North America
Essen BioScience Inc.
300 West Morgan Road
Ann Arbor, Michigan, 48108
Telephone +1 734 769 1600
E-Mail: AskAScientist@sartorius.com
Online Store: shop.intellicyt.com

Europe
Essen BioScience Ltd.
Units 2 & 3 The Quadrant
Newark Close
Royston Hertfordshire
SG8 5HL
United Kingdom
Telephone +44 (0) 1763 227400
E-Mail: euorders.UK03@sartorius.com

APAC
Essen BioScience K.K.
4th floor Daiwa Shinagawa North Bldg.
1-8-11 Kita-Shinagawa
Shinagawa-ku, Tokyo
140-0001
Japan
Telephone: +81 3 6478 5202
E-Mail: orders.US07@sartorius.com