APPLICATION NOTE

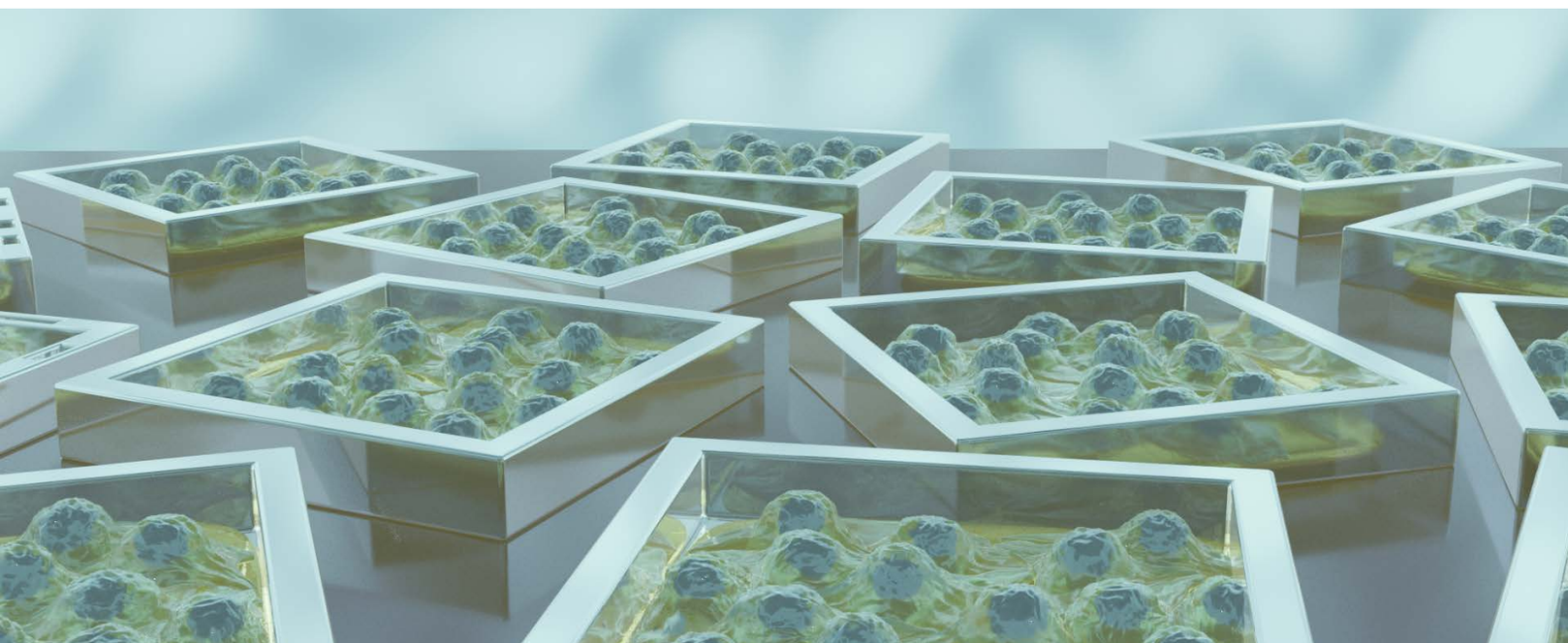
semarion

Accelerating Transient Transfection Assays

Cryopreserved, Transfected, and Assay-ready Adherent Cells on SemaCyte® Microcarriers

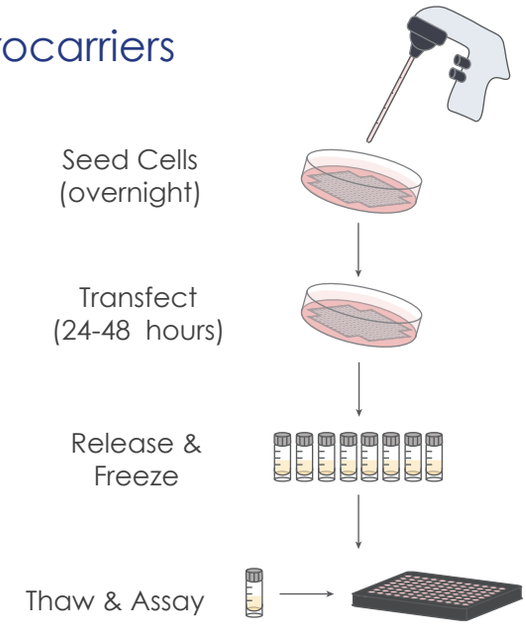
SemaCyte® microcarriers enhance drug discovery workflows by enabling the cryopreservation of large batches of assay-ready adherent cells, significantly reducing preparation time. In this application, A549 cells transfected with a p53:MDM2 NanoBRET pair were cryopreserved and recovered without loss of viability or transfection efficiency. The SemaCyte® technology accelerated the assays by up to 8-fold, eliminating the need for a 2-3 day cell preparation step, offering more flexible and efficient screening campaigns.

Flexibility: Decouple cell preparation and assaying
Reliability: Batch produce assay-ready cells to ensure uniformity
Throughput: Thaw, dispense, and assay within 1 hour



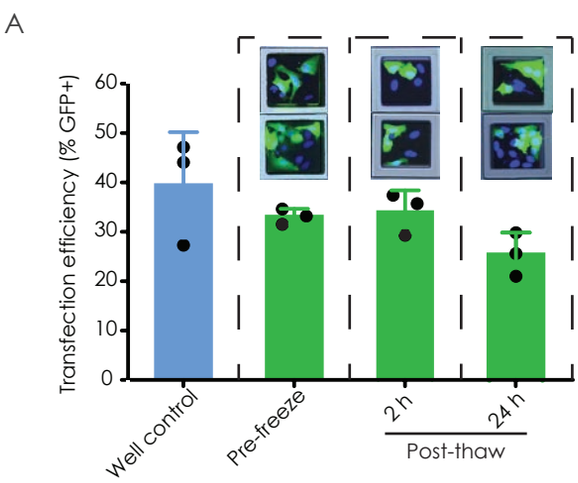
NanoBRET Assays with SemaCyte® Microcarriers

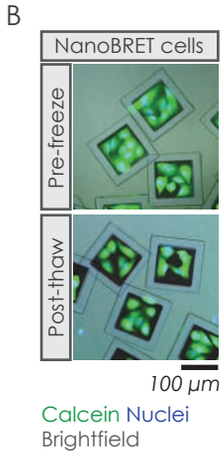
To perform NanoBRET assays using SemaCyte® microcarriers, cells are seeded onto the SemaCyte® Seeding Dish (SD20), which contains 50,000 microcarriers. After reaching the desired confluency, cells are transfected (e.g. with Lipofectamine 3000) and incubated for 24-38 hours. The microcarriers are then released, aliquoted, and cryopreserved. Upon thawing, the cells maintain high transfection efficiency, adherent morphology, and local confluency, ready to be directly dispensed into microplates with compounds for immediate assay use.

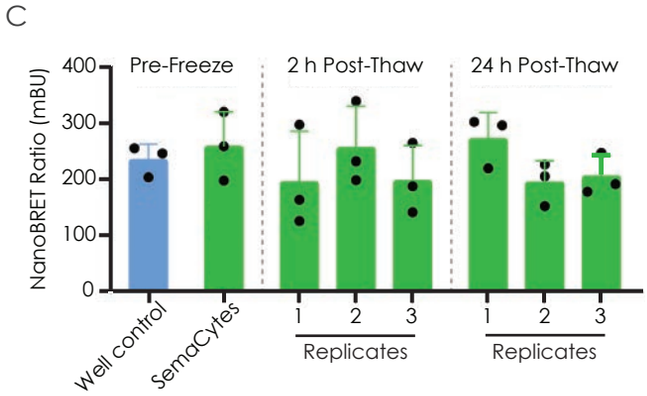


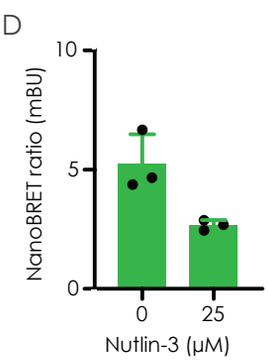
Adherent cells transfected, cryopreserved, and available to assay in minutes

Assay-Ready NanoBRET A549 Cells Transfected with a p53:MDM2 Pair









(A) GFP transfection had an efficiency of 30-40% before and after freezing.
(B) A549 cells were transfected with a p53:MDM2 NanoBRET Protein-Protein Interaction pair and frozen for later use. Transfected cells displayed high levels of cell viability post-thaw.
(C) 50 SemaCytes per well were dispensed into 384-well plates and NanoBRET ratios measured with the BMG FLUOstar Omega. Ratios were consistent and stable 2 and 24 hours after thawing.
(D) Nutlin-3 was added 1 hour after thawing and incubated for 4 hours to measure its interference with the p53:MDM2 interaction.