SEP 23, 2023

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Rapalog-induced chemical dimerization experiments

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ABSTRACT

This protocol describes Rapalog-induced chemical dimerization experiments.

ATTACHMENTS

760-1928.pdf

MATERIALS

Reagents

iDimerize™ Inducible Heterodimer System **Takara Bio Inc. Catalog #635067**

LSR Fortessa Cell Analyzer (BD Biosciences)

DOI: dx.doi.org/10.17504/protocols.io.n92ldmyynl5b/v1

Protocol Citation: Elias Adriaenssens 2023. Rapalog-induced chemical dimerization experiments. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldmyynl5b/v1

Protocol status: Working
We use this protocol and it's working

Created: Jun 28, 2023

Last Modified: Sep 23, 2023

PROTOCOL integer ID: 84159

Keywords: Rapalog-induced chemical dimerization experiments

Rapalog-induced chemical dimerization experiments

1d

1 To perform chemical-induced dimerization (CID) experiments we will first establish cell lines expressing the FRB-Fis1 and FKBP-GFP-GOI (FKBP-GFP fused to our gene of interest).

2 HeLa cells are consecutively transduced with lentivirus for FRB-Fis1, which also expresses mitochondrially targeted monoKeima (mt-mKeima), and then with FKBP-GFP-GOI. See the lentivirus transduction protocol for further details.

3 Once cell lines are established, cells are seeded in 6-well plates the day before the experiment (density: 800k per well).

4 Induce FKBP-FRB dimerisation by treating the cells with [M] 50 nanomolar (nM) Rapalog A/C hetero-dimerizer rapalog (635057, Takara) for 24:00:00. 1d

5 After 24h, collect cells by trypsinization and take the cells to the FACS facility for mt-mKeima analysis.

6 Analyze the cells by flow cytometry, using the mt-mKeima ratio (561/405) as a readout with an LSR Fortessa Cell Analyzer (BD Biosciences).

7 Gate the cells for GFP/mt-mKeima double-positive cells, and collect 10,000 events for this population.

8 Analyze data using FlowJo.