Multiplexing Cell-Based Assays: Get More Biologically Relevant Data

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Multiplexing assays for more informative data

- Plate-based assays for viability, cytotoxicity and apoptosis measurement
- Using multiplex assays to understand cell death mechanism
- Monitoring cell response in multiple applications
Distinct enzyme chemistries allow multiplexing in the Dual-Luciferase® Assay

1. Determine promoter response element (RE) of interest.
2. Clone RE upstream of the firefly luciferase (firefly) gene.
3. Transfect construct into cells.
4. Add luciferase detection reagent and measure firefly luciferase activity.
5. Add Renilla detection reagent and measure Renilla luciferase activity. Note: Reduced luminescence in well “A” may be due to cytotoxicity.

The Stop & Glo® Reagents quench firefly luminescence and provide the substrate for Renilla luciferase

Control Renilla corrects for:
- cell death
- bad transfection
Understand overall cellular response to treatment

If only reporter activity is measured, the impact of compound treatment on cell health may be overlooked.
Live/Dead assays easily added upstream

- Treat cells in white or black wall plates.
- Incubate 30 minutes.
- Add CellTiter-Fluor, CytoTox-Fluor, or MultiTox-Fluor Reagent.
- Measure AFC & R110 fluorescence.
- Perform 2nd assay using normal protocol.

OPTIONS:
- ONE-Glo™ Reporter Assay
- Bright-Glo™ Reporter Assay
- Steady-Glo® Reporter Assay
- Renilla-Glo™ Reporter Assay
- Beta-Glo® Reporter Assay
- Caspase-Glo® 3/7 Assay
- Caspase-Glo® 8 Assay
- Caspase-Glo® 9 Assay
- ApoONE® Caspase-3/7 Assay*
- HDAC-Glo™ I/II Assay (coming soon)
- GSH-Glo™ Glutathione Assay
- P450-Glo™ Cell-Based Assays
- CellTiter-Glo® Assay
- CytoTox-ONE™ Assay
**Normalize Reporter Data to viable cells**

**Bright-Glo™ Luciferase Assay**
- Bright-Glo Only
  - CV = 58%
- GF-AFC Normalized
  - CV = 8%

**Steady-Glo® Luciferase Assay**
- Steady-Glo Only
  - CV = 80%
- GF-AFC Normalized
  - CV = 8%

**GloResponse™**
NF-κB Cells at 5K to 25K cells/well
Monitor changes in GSH levels in cells under stress

A change in GSH levels is important in assessment of toxicological responses and is an indicator of oxidative stress, potentially leading to apoptosis or cell death.
Multiplexing Viability and GSH Levels

Add MultiTox-Fluor Reagent

Incubate 30 min

Record Fluorescence

Remove medium (GSH background)

Add GSH-Glo Reagent, 30 min

Add Luciferin Detection Reagent, 15 min

Record Luminescence

Viability EC$_{50}$ = ND
Cytotoxicity EC$_{50}$ = ND
Glutathione EC$_{50}$ = 188nM

Again, timing is everything.
In this window, there is no change in viability but be assured, a lack of GSH will lead to cytotoxicity.
Histone Deacetylases are cancer targets

- Acetylated histones have tight structure, restricting transcription
- Deacetylated histones have a loose structure, encouraging transcription
- Balance of acetylation and deacetylation is important & an imbalance is not good.

Hess-Stumpp et. al. 2007
Help identifying on- and off-target effects

Multiplexed measurement of HDAC inhibitor activity with HDAC-Glo™ I/II and cytotoxicity with Multitox-Fluor TM Reagent clearly delineates cell lines that are sensitive to HDAC inhibitors. Exposure of two cell lines to apicidin for 24h followed measurement identified the HDAC inhibitor sensitive cell line and showed good correlation between HDAC inhibition and cytotoxicity.

No apparent cytotoxicity

Cytotoxicity
What multiplexing can do for you

Multiplexing gives a more complete picture of what’s happening in the cell

- Reduces ambiguity
- Eliminates variables
- Normalizes data
- Increases content
Monitoring the cellular response improves data

Cell health monitoring is easily added upstream of many Promega cell-based assays.

- ApoTox-Glo™ Triplex Assay
- MultiTox-Fluor Multiplex Cytotoxicity Assay
- CytoTox-Fluor™ Cytotoxicity Assay
- CellTiter-Fluor™ Cell Viability Assay

- ONE-Glo™ Reporter Assay
- Bright-Glo™ Reporter Assay
- Steady-Glo® Reporter Assay
- Renilla-Glo™ Reporter Assay
- Beta-Glo™ Reporter Assay

- Caspase-Glo® 3/7 Assay
- Caspase-Glo® 8 Assay
- Caspase-Glo® 9 Assay
- ApoONE™ Caspase-3/7 Assay*

- HDAC-Glo™ I/II Assay (coming soon)
- GSH-Glo™ Glutathione Assay
- P450-Glo™ Cell-Based Assays

- CellTiter-Glo® Assay
- CytoTox-ONE™ Assay

* works with CellTiter-Fluor Assay only

Other multiplexing possibilities exist. Ask Technical Services.
Need help?

Promega Technical Services

- Experienced & highly trained scientists
- Varied technical expertise
- Varied scientific expertise

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