HTS384 Screening Methodology

The NCI-60 HTS384 screen is a modernized, fully automated, high-throughput assay used to evaluate the anti-proliferative effects of small molecules across 60 human tumor cell lines. Replacing the legacy 96-well format, the current 384-well plate format uses fewer cells, longer exposure times, and a CellTiter-Glo® luminescent readout to more accurately reflect compound potency and tumor specificity.

This platform is used for all current screening submissions and offers significant improvements in throughput, sensitivity, reproducibility, and data comparability.

Assay Format

Feature	HTS384 (Current)
Plate Format	384-well
Assay Type	Cell viability / cytotoxicity
Readout	CellTiter-Glo (luminescent ATP assay)
Exposure Duration	72 Hours (3 days)
Incubation Conditions	37°C, 5% CO_2, humidified
Compound Dosing	5-point dose-response

Assay Workflow

1. **Seeding:**
   Cells from each of the 60 tumor cell lines are seeded into 384-well plates at optimal densities depending on doubling time.

2. **Compound Addition:**
   Each compound is tested in five concentrations, typically using a log10 dilution series. Compounds are solubilized in DMSO and diluted to working concentrations in complete growth medium.

3. **Incubation:**
   After compound addition, plates are incubated for 120 hours (5 days), allowing adequate time to detect effects on cell proliferation and viability.

4. **Detection with CellTiter-Glo®:**
   After incubation, CellTiter-Glo reagent is added directly to wells. This reagent lyses the cells and generates a luminescent signal proportional to intracellular ATP, which correlates with the number of viable cells.

5. **Signal Measurement:**

Luminescence is measured using an automated plate reader. Data is normalized to controls and used to calculate dose-response metrics.

Data Analysis Metrics

Each compound is evaluated based on three standard pharmacodynamic parameters:

- **GI50** (Growth Inhibition 50%):
  Concentration at which cell growth is inhibited by 50% compared to untreated control.

- **TGI** (Total Growth Inhibition):
  Concentration at which cell number remains unchanged from baseline (net zero growth).

- **LC50** (Lethal Concentration 50%):
  Concentration at which 50% of the cell population is killed (net cytotoxicity).

These values are interpolated from the concentration/growth curves for each of the 60 cell lines. These results are reported via interactive mean graphs and can be analyzed using the COMPARE tool for mechanistic insights.

Compatibility with Historical Data

For many mechanisms of action, HTS384 screening results show strong concordance with the legacy NCI-60 assay results. The use of modern luminescence-based detection retains the core comparative features needed to:

- Analyze trends over time
- Use COMPARE on legacy vs. new data
- Support continuity across research studies

Read: Cancer Res. 2024 Aug 1;84(15):2403–2416
High-throughput screening of the NCI-60 human tumor cell line panel using a modernized ATP-based luminescence assay

Additional Resources:

- Submit a Compound for Screening

- COMPARE Tool for Mechanism of Action Prediction

- Download Sample Data Sets