Classic NCI-60 Screen (Archived)

This section documents the original sulforhodamine B (SRB)-based methodology used prior to the HTS384 modernization. The classic format is no longer in use for new compound evaluations.

Overview of Legacy Assay

- **Format:** 96-well microtiter plates
- **Detection Method: Sulforhodamine B (SRB)** protein stain
- **Exposure Time:** 48 hours
- **Cell Viability Measurement:** Colorimetric absorbance at 515 nm
- **Metric Calculations:** GI50, TGI, and LC50 (based on protein content)

Protocol Summary

1. **Seeding:**
   Cells were plated in 96-well format and incubated for 24 hours before drug addition.

2. **Compound Addition:**
   Compounds were added in five concentrations via serial dilution, followed by a 48-hour incubation.

3. **Fixation & Staining:**
   Cells were fixed with trichloroacetic acid (TCA), stained with SRB dye, and washed to remove unbound dye.

4. **Quantification:**
   Bound stain was solubilized and read at 515 nm. Absorbance reflected total protein content, correlating with cell number.

5. **Data Analysis:**
   The same three metrics (GI50, TGI, LC50) were computed based on optical density measurements and time-zero controls.

Historical Use

- The SRB-based assay was the standard from 1990 to 2023.
- Its results underpin decades of data in the COMPARE algorithm and historical NCI publications.
- Many compounds tested under this system still form the foundation of mechanism of action libraries.