QUICK GUIDE
Designing a Service Study with INDIGO

Study Work Order (SWO)

An electronic SWO must be completed and returned to INDIGO before a study can be initiated. SWO files (Excel) may be obtained through INDIGO's website or Customer Service Representative.

General Considerations for Assay Design

- Low throughput screening: Assays are performed in 96-well plates with n ≥ 3 replicates per test concentration.
- Agonist-mode assays: Assays include one INDIGO reference agonist. Additional reference agonists may be included but are subject to the same per well charge rate as used for test compounds.
- Antagonist-mode assays: These include one reference antagonist if one is commercially available and validated by INDIGO. Additional reference antagonists are subject to the same per well charge rate as used for test compounds.
- Compound test concentrations: Treatment concentrations are prepared at INDIGO using serial dilution in fixed increments; the starting treatment concentration and increment of dilution are specified on the SWO by the client (refer to Table 1 as a guide).
- EC50 & IC50: Accurate determinations of EC50 and IC50 values necessitate establishing upper and lower plateaus on a sigmoid curve-fit of the dose-response data. Therefore, it is then important to analyze a broad range of test concentrations. At a minimum, we recommend spanning a 5,000-fold concentration range over 8 doses. This strategy requires the use of 3.33-fold or 4-fold increments of serial dilution (see Table 1). INDIGO's recommendation for EC50 and/or IC50 determinations: a minimum of 7 test concentrations.
- Cytotoxicity analyses: Cyto-tox analyses are important when conducting antagonist mode, or inverse-agonist mode assays. Compounds that induce a cytotoxic response will produce false-positive results in assays that measure loss of signal in response to compound treatments.

Provide Test Compound Stocks 'Assay Ready'

- All compound stocks should be prepared using a single solvent, preferably DMSO. Please inquire regarding the use of alternative organic solvents. Aqueous stock solutions may be submitted but they must be filter sterilized and devoid of preservatives and chelating agents.
- Compound stocks should be at a uniform concentration and arrayed in 96-well format. DMSO stocks should be between 250x-1,000x-concentration relative to the highest treatment concentration; eg., provide 10 mM stocks when the highest assay concentration is to be 10 µM would be considered 1,000x. Aqueous stocks may be provided at no lower that 10x-concentration relative to the highest treatment concentration.
- Minimum volumes: 75 µl of 1,000x-concentrated stock solutions; or 500 µl of 10x-concentrated aqueous stock solutions.
- Sample handling charges will be applied for processing dry compounds. 5 mg or less of dry compounds, as well as an exact weight of material, should be provided. Do not send excess quantities. INDIGO does not perform powder transfers or the weighing of dry test compounds.
- Additional charges will be applied if extensive sample handling is required to generate 'assay ready' stocks (e.g., the manual transfer of compound stocks from individual vials into a 96-format array; manual adjustments of concentrations to achieve uniform stock concentrations; filter-sterilization of aqueous stocks; sonication of stocks to gain solubility or establish homogenous solutions; other manipulations).

Table 1. Example Schemes for the Serial-Dilution of Test Compounds

Dilution Increment ↓	Number of Serial Dilutions of Test Compound										Number of Serial Dilutions of Test Compound (nM)
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	
3-fold	10,000	3,333	1,111	370	123	41.2	13.7	4.57	1.52	0.508	
3.33-fold (half-Log)	10,000	3,003	902	271	81.3	24.4	7.33	2.20	0.661	0.199	
4-fold	10,000	2,500	625	156	39.1	9.77	2.44	0.610	0.153	0.0381	
5-fold	10,000	2,000	400	80.0	16.0	3.20	0.640	0.128	0.0256	0.00512	

↑ Example Highest Treatment Conc., nM

Minimum recommendation for EC50 and/or IC50 determinations:

- 9 test concentrations generated in 3.0- or 3.33-fold dilutions, *or*
- 8 test concentrations generated in 3.33- or 4-fold dilutions, *or*
- 7 test concentrations generated in 4-fold or 5-fold dilutions

Values highlighted in green correspond to a recommended ≥5,000-fold span in test conc.