

A High Throughput Screening Assay for Hepatotoxicity Using Cryopreserved Human Hepatocytes

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Focus on HTS Toxicology

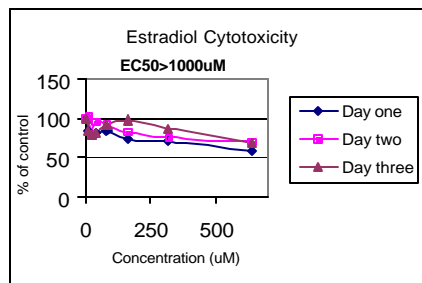
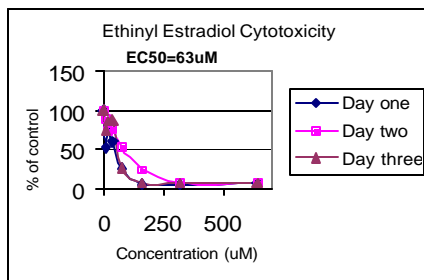
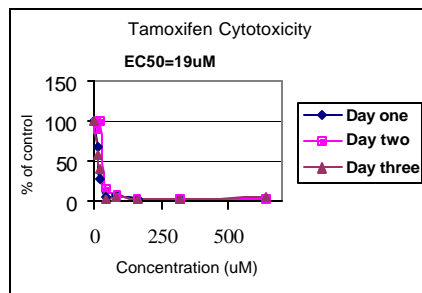
- Appropriate species - human
- Appropriate organ - liver
- Early toxicity screening of drug candidates avoids toxicity "surprises"

HTS Cytotoxicity Protocol (ATP Assay)

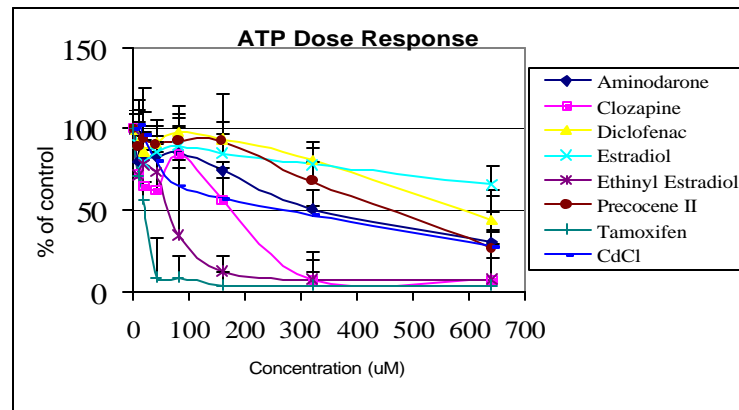
- T=0: Add 10 uL of 2X test chemicals and 2,000 hepatocytes in 10 uL to 384-well plate (per well).
- Incubate 2 hr at 37°C, 5% CO₂.
- T=2 hr: Add 10 uL lysis buffer, wait 2 minutes.
- T=2 hr + 2 min: Add 10 uL luciferin/luciferase substrate, wait 10 minutes.
- T=2 hr + 12 min: Measure luminescence of each well.
- Reagent cost: \$0.20/well (cells+ATP reagents).
- Comment: Reduced cost per well and higher throughput.

Cytotoxicity	EC50 (uM)
Aminodarone	382
Clozapine	136
Diclofenac	407
Estradiol	>1000
Ethinyl Estradiol	63
Precocene II	354
Tamoxifen	19
CdCl	207

ATP Day-to-Day Variations



HTS ATP Assay in 384-well



384-Well ATP Assay Variability (Mean % CV, n=6)								
Concentration (uM)	Aminodarone	Clozapine	Diclofenac	Estradiol	Ethinyl Estradiol	Precocene II	Tamoxifen	CdCl
0	11.5	10	10	10	10	10	11.5	12
10	16	17	11	21	25	16	8	15
20	16	29	15	8	20	32	12	14
40	7	23	10	10	18	15	25	12
80	16	17	16	18	46	17	14	12
160	6	20	28	12	10	12	9	12
320	12	18	11	10	12	12	9	5
640	27	29	18	11	13	22	26	10

Summary: HTS Cytotoxicity

- Cryopreserved human hepatocytes are now available as a standardized reagent.
- A high-throughput toxicity assay has been established using ATP as an endpoint.
- Assay can be performed in 384-well plates at a reagent cost of \$0.20/well.
- We are seeking external collaborators to submit compounds with known in vivo toxicity to develop large database.