US EPA ARCHIVE DOCUMENT

Parameters of Assays used in Development of ER Expert System

Table 1. Trout ER Binding Assay Parameters

Protocol	**Rainbow Trout Liver Cytosol**
Assay type	competitive binding - [3H]E2
Receptor Source	rainbow trout liver cytosolic fraction
Receptor	Endogenous trout ERs
Kd (nM)	2.3
Bmax (fmol/mg)	20.9
total protein in assay tube (mg/ml)	4.5
Reference assay activity: E2 IC50 (nM)	5
Detection Method	Separation of bound & free [3H]-E2
Assay format	Test tube
[3H]-E2 (nM)	5
Carrier	Ethanol preferred; Alternatively can use DMSO or methanol
Max Conc.	1.5%
Control	Yes
Vehicle Control reps	2
Reference Control	E2
Reference Conc.	0.1, 1, 10, 100, and 1000 nM
replicates	2
replicate runs	Duplicate or triplicate in parallel with test chemical
Test chemical	
Stock Conc.	1.285 M or to solubility
Test Chemical Range	10 to 10,000 µM
Test Chem Replication	2/conc./run; 2 runs at minimum
Assay Buffer pH	7.6
Assay Temp	4°C
Incubation period	20h
Solubility	Checked visually in stock chemical and in cytosol
Analytical	LC-MS and GC-MS on select chemicals from each chemical class

US EPA ARCHIVE DOCUMENT

Table 2. Vitellogenin mRNA Expression Assay Parameters using Trout Liver Slices

Assay	Slice Vtg mRNA AGONIST	Slice Vtg mRNA ANTAGONIST
Assay type	mRNA	mRNA
Assay Mode	Agonist	Antagonist
Assay Tissue	freshly prepared liver slices	freshly prepared liver slices
Cell/Tissue Origin	Immature male rainbow trout liver	Immature male rainbow trout liver
Receptor (s)	all endogenous types	all endogenous types
Endogenous Gene	Vitellogenin	Vitellogenin
Endpoint	Q-PCR	Q-PCR
data as	Vtg mRNA copy number	Vtg mRNA copy number
expressed & graphed as	Vtg mRNA copy number per 400 ng total RNA	Vtg mRNA copy number per 400 ng total RNA
Carrier	Ethanol (EtOH)	Ethanol (EtOH)
Carrier Conc.	0.2%	0.2%
Carrier Control	Yes	yes
Reference Control	E2	E2
No. of Reference Concentrations	6 pt curve; n=2 slices per concentration (-10 to -5 logM; 0.1 nM to 10 µM)	6 pt curve; n=2 slices per concentration (-10 to -5 logM; ; 0.1 nM to 10 µM)
Cell Viability	1. LDH is primary cytotox test; MTT on a select group of chemicals. 2. Slice wet weight, RNA yield and integrity used as secondary cytotox indicators. 3. Also monitor pH, osmolality, K^+, lactose	1. LDH is primary cytotox test; MTT on a select group of chemicals. 2. Slice wet weight, RNA yield and integrity used as secondary cytotox indicators. 3. Also monitor pH, osmolality, K^+, lactose
Test Chemical		
Stock Concentration in EtOH	Target of 3.67M for maximum, Actual varies and is chemical specific	Target of 3.67M for maximum, Actual varies and is chemical specific
Test Chemical Conc. Range (M)	Chemical specific, often to solubility limit	Chemical specific, often to solubility limit
Test Chem Conc. Response curve.	6 test concentrations	6 test concentrations in combination with at least two E2 concentrations separated by 2logM
Sample Replication	6 reps per chemical concentration	5 reps per chemical concentration
Test Replication	A minimum of 2 separate tests	A minimum of one antagonist
Solubility	By visual observation and nephelometer	By visual observation and nephelometer
Analytical	Performed on limited number of test chemicals, often to confirm metabolism of test chemical	Performed on limited number of test chemicals, often to confirm metabolism of test chemical
Plate Format	12-well	12-well
Tissue per well	1 precision cut liver slice/well; 8mm dia. by 200 µm thick;	1 precision cut liver slice/well; 8mm dia. by 200 µm thick;
Exposure Duration	48h at 11°C	48h at 11°C
media pH	7.6	7.6
Do slices have metabolic activity?	yes	Yes

US EPA ARCHIVE DOCUMENT