Anticonvulsant Screening Program

Test 76 Results - In-vitro Hippocampal Slice Culture Neuroprotection Assay (NP)

ASP ID: 1 **U** **Screen ID: 1**

Solvent Code: DMSO **Solvent Prep:**

Test Date: 12-Oct-2009

Reference: 439:220

Summary of NP Assay: NMDA

- **Test Result:** No Neuroprotection
- **ADD compounds evaluated:** 1 000004

Note: This experiment is run at two different concentrations of candidate drug against a fixed concentration of excitotoxin. If multiple candidates from the same participant source are scheduled for NP screening we will test compounds in pairs whenever possible.

Comments:

TEST 76: *in vitro* HIPPOCAMPAL SLICE CULTURE NEUROPROTECTION ASSAY

Compound 1 : ADD Number: 000001 **Batch:** **Date Started:** 12-Oct-2009

Compound 2 : ADD Number: 4 **Batch:** **Date Completed:** 14-Oct-2009

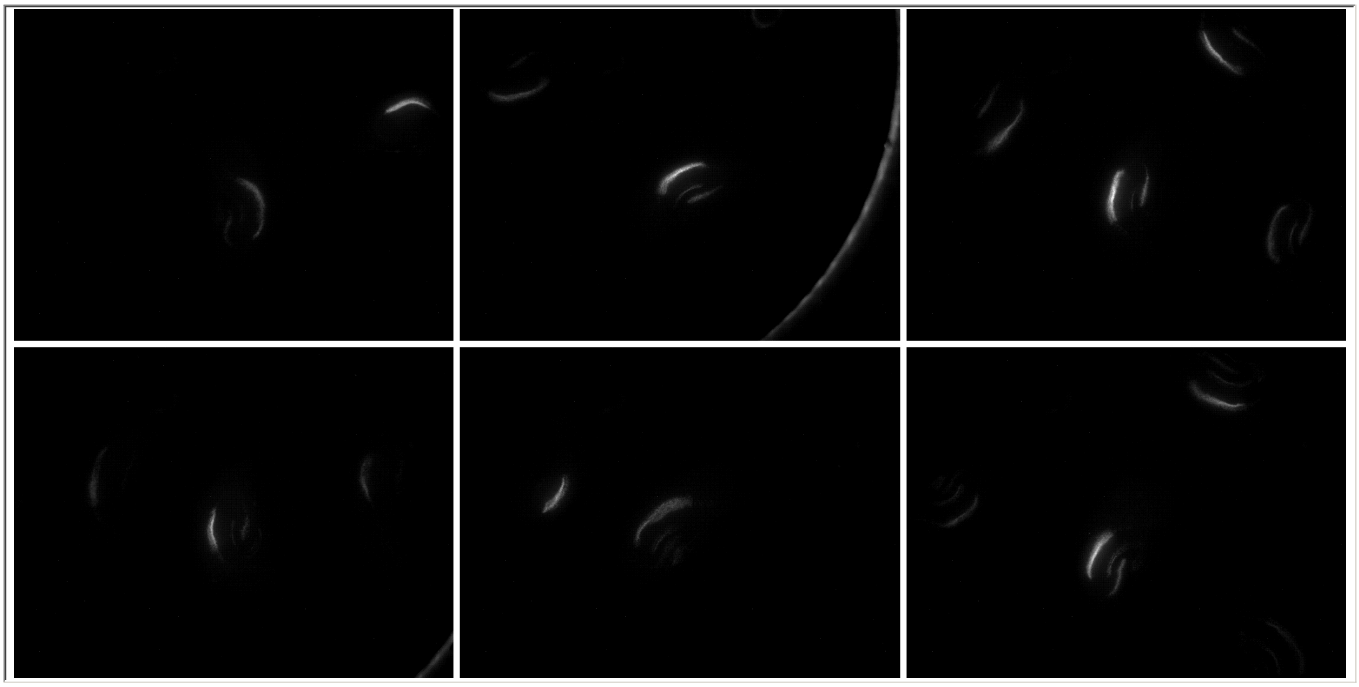
References: 439: 220

Excitotoxin: NMDA **Insult Duration:** 4 Hours **Solvent:** DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

A1	NMDA 10µM	**A2**	NMDA 10µM + 000001 10µM + 000004 10µM	**A3**	NMDA 10µM + 000001 10µM + 000004 10µM



B1	NMDA 10µM	**B2**	NMDA 10µM + 000001 100µM + 000004 100µM	**B3**	NMDA 10µM + 000001 100µM + 000004 100µM

PRIMARY SCREEN EXPERIMENT DESCRIPTION

The "Primary Screen Experiment" is a qualitative assessment of the ability of a compound to prevent excitotoxic cell death. Organotypic hippocampal slice cultures are treated with N-methyl-D-aspartate (NMDA) or kainic acid (KA) to induce neuronal cell death. Propidium iodide (PI), a membrane-impermeant compound, is included in all wells of the culture plate. Dying cells have compromised cell membranes, thus PI may diffuse into the cell, intercalate with DNA and fluoresce. Thus, the intensity of the PI fluorescence is proportional to the amount of cell death in the individual slices. Hippocampal slice cultures are treated with the excitotoxin alone, or where indicated above, with the excitotoxin and either **one or two** investigational compounds at the concentrations indicated. If neuroprotection occurs as a consequence of the added compound, slice cultures will have a visibly reduced fluorescent intensity when compared to the slice cultures that have been treated with the excitotoxin alone.

Anticonvulsant Screening Program

Test 76 Results - In-vitro Hippocampal Slice Culture Neuroprotection Assay (NP)

ASP ID: 1 **U** **Screen ID: 2**

Solvent Code: **DMSO** **Solvent Prep:**

Test Date: **12-Oct-2009**

Reference: **439:220,229,234**

Summary of NP Assay: **Kainic acid**

- **Test Result:** **Neuroprotective effect**

Response

Excitotoxin: **Kainic acid**

ASP Compound Conc.(uM)	# of slices	% of Total Propidium Iodide Uptake (mean +/- S.E.M)	
0	15	48.6 +/- 3.3	☐
10	7	45.0 +/- 2.7	☐
30	8	42.6 +/- 4.1	☐
100	7	32.3 +/- 4.0	☑
300	8	30.1 +/- 4.1	☑

Note: box is checked if data is significantly different from excitotoxin treatment alone, p<0.05.

IC50(uM): **> 300.00 +/-** **S.E. M**

Comments:

TEST 76: *in vitro* HIPPOCAMPAL SLICE CULTURE NEUROPROTECTION ASSAY

Compound 1 : **ADD Number:** 000001 **Batch:** **Date Started:** 12-Oct-2009

Compound 2 : **ADD Number:** **Batch:** **Date Completed:** 30-Oct-2009

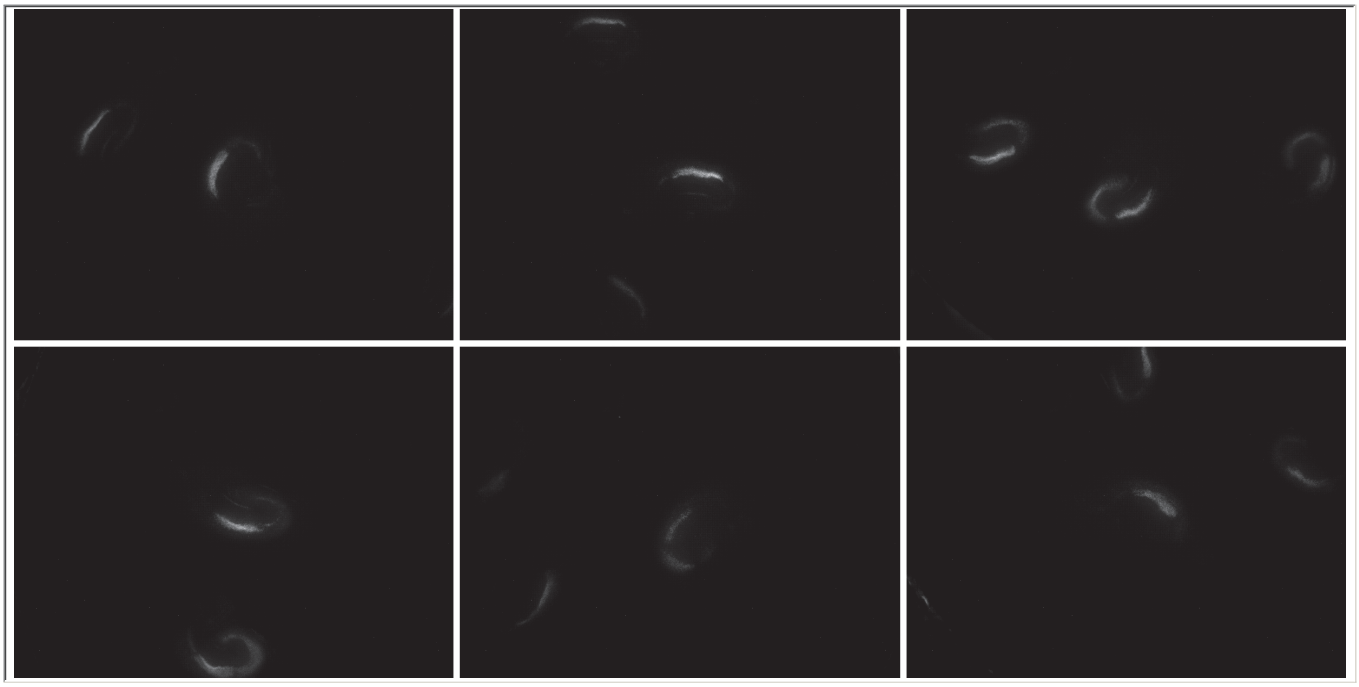
References: 439: 220, 229, 234

Excitotoxin: **Kainic Acid** **Insult Duration:** 4 Hours **Solvent:** DMSO

Primary Screen Results: **Neuroprotection observed**

EXPERIMENT IMAGES & WELL DESCRIPTION

A1	KA 20µM	**A2**	KA 20µM + 000001 10µM	**A3**	KA 20µM + 000001 10µM



B1	KA 20µM	**B2**	KA 20µM + 000001 100µM	**B3**	KA 20µM + 000001 100µM

PRIMARY SCREEN EXPERIMENT DESCRIPTION

The "Primary Screen Experiment" is a qualitative assessment of the ability of a compound to prevent excitotoxic cell death. Organotypic hippocampal slice cultures are treated with N-methyl-D-aspartate (NMDA) or kainic acid (KA) to induce neuronal cell death. Propidium iodide (PI), a membrane-impermeant compound, is included in all wells of the culture plate. Dying cells have compromised cell membranes, thus PI may diffuse into the cell, intercalate with DNA and fluoresce. Thus, the intensity of the PI fluorescence is proportional to the amount of cell death in the individual slices. Hippocampal slice cultures are treated with the excitotoxin alone, or where indicated above, with the excitotoxin and either **one or two** investigational compounds at the concentrations indicated. If neuroprotection occurs as a consequence of the added compound, slice cultures will have a visibly reduced fluorescent intensity when compared to the slice cultures that have been treated with the excitotoxin alone.