

Anticonvulsant Screening Program

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

ASP ID: 299055 U Screen ID: 2

Solvent Code: DMSO Solvent Prep:

Test Date: 22-Apr-2009

Reference: 439:87,91,94

Summary of NP Assay: NMDA

● Test Result: No Neuroprotection

Comments:

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

Compound 1 : ADD Number: 299055 Batch: U Date Started: 22-Apr-2009

Compound 2 : ADD Number: Batch: Date Completed: 08-May-2009

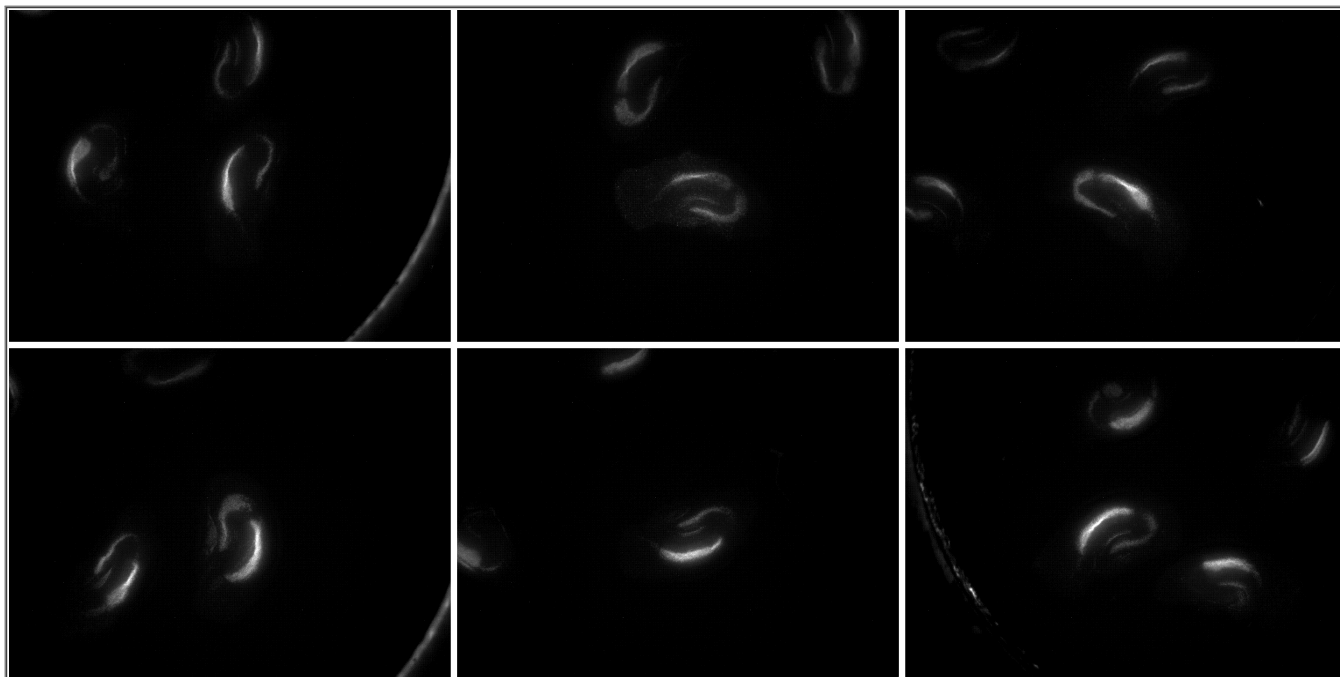
References: 439: 87, 91, 94

Excitotoxin: NMDA Insult Duration: 4 Hours Solvent: DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

A1	A2	A3
NMDA 10µM	NMDA 10µM +	NMDA 10µM +
	299055 10µM	299055 10µM



B1	B2	B3
NMDA 10µM	NMDA 10µM +	NMDA 10µM +
	299055 100µM	299055 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: 299055 U Screen ID: 3

Solvent Code: DMSO Solvent Prep:

Test Date: 28-Jan-2009

Reference: 439:25-43

Summary of NP Assay: Kainic acid

● Test Result: Neuroprotective effect

Comments:

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

Compound 1 : ADD Number: 299055 Batch U Screen ID: 3 Date Started: 28-Jan-2009

Compound 2 : ADD Number: Batch Screen ID: Date Completed: 20-Feb-2009

References: 439: 25, 31, 37, 43

Excitotoxin: KA

Insult Duration: 4 Hours

Solvent: DMSO

Primary Screen Results: Neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

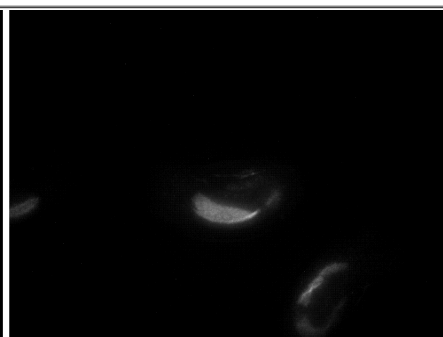
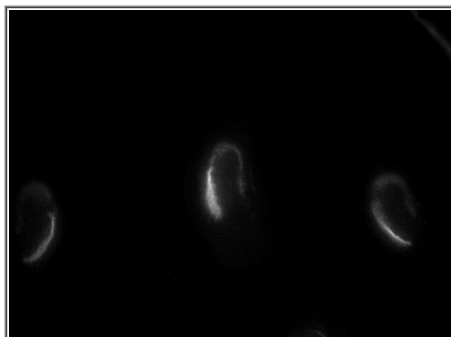
A1 KA 20µM

A2 KA 20µM +

A3 KA 20µM +

299055 10µM

299055 10µM



B1 KA 20µM

B2 KA 20µM +

B3 KA 20µM +

299055 100µM

299055 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: 299055 U Screen ID: 4

Solvent Code: DMSO Solvent Prep:

Test Date: 28-Jan-2009

Reference: 439:25,31,37,43

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	16	56.4 +/- 2.4	☐
10	8	51.2 +/- 3.7	☐
30	8	30.7 +/- 1.6	☒
100	7	34.9 +/- 3.3	☒
300	7	25.4 +/- 3.8	☒

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

IC50(uM): 336.10 +/- 3.52 S.E. M

Comments:

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

Compound 1 : ADD Number: 299055 Batch: U Date Started: 28-Jan-2009

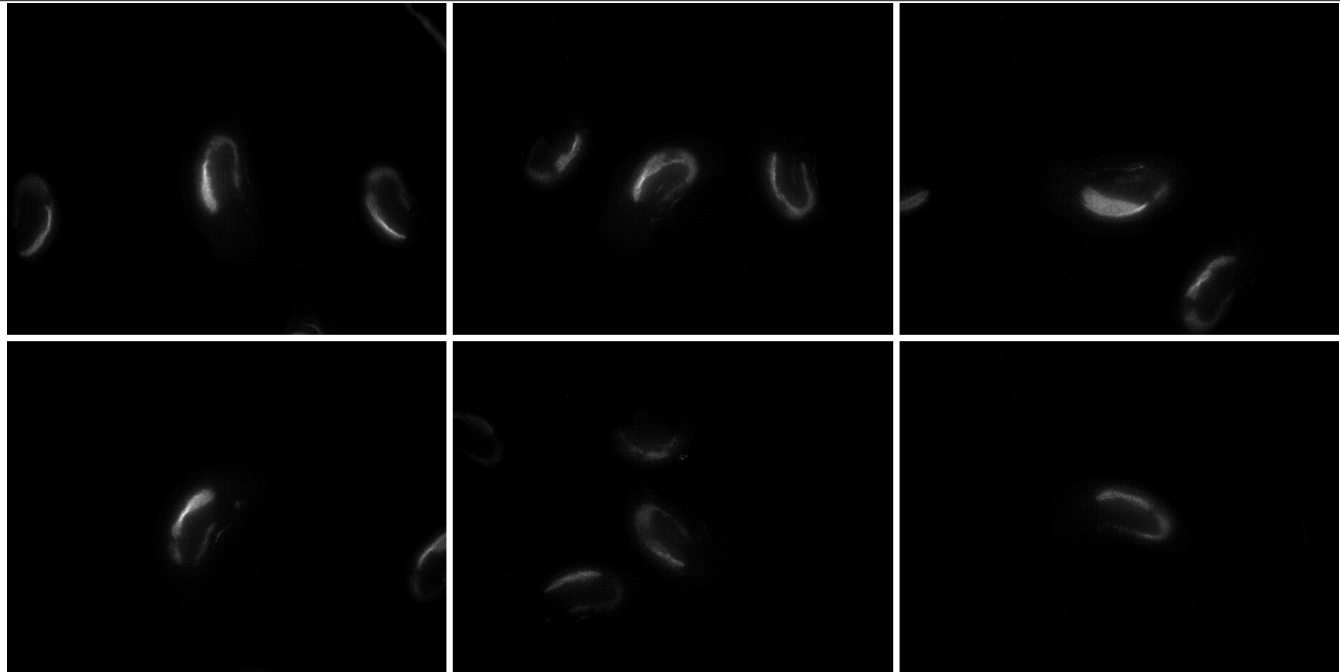
Compound 2 : ADD Number: Batch: Date Completed: 20-Feb-2009

References: 439: 25, 31, 37, 43

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

Primary Screen Results: Neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

A1	KA 20µM	A2	KA 20µM +	A3	KA 20µM +
			299055 10µM		299055 10µM
					
B1	KA 20µM	B2	KA 20µM +	B3	KA 20µM +
			299055 100µM		299055 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.