

Anticonvulsant Screening Program

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

ASP ID: 7 U Screen ID: 1

Solvent Code: DMSO Solvent Prep:

Test Date: 26-May-2010

Reference: 450:186

Summary of NP Assay: NMDA

- Test Result: No Neuroprotection
- ADD compounds evaluated: 7 417023

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: 000007

Batch: U

Date Started: 26-May-2010

Compound 2 : ADD Number: 417023

Batch: U

Date Completed: 28-May-2010

References: 450: 186

Excitotoxin: NMDA

Insult Duration: 4 Hours

Solvent: DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

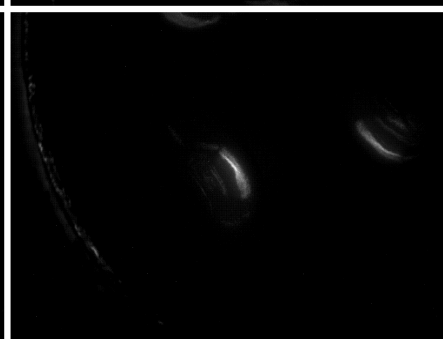
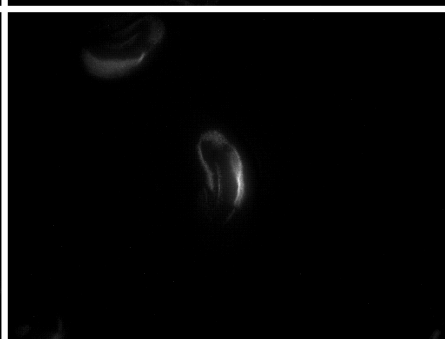
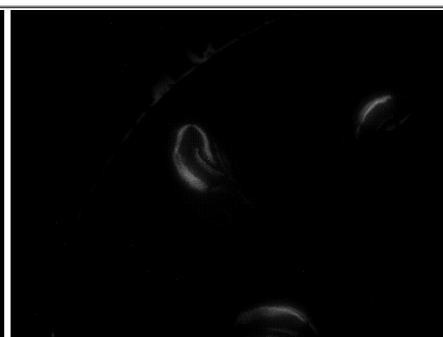
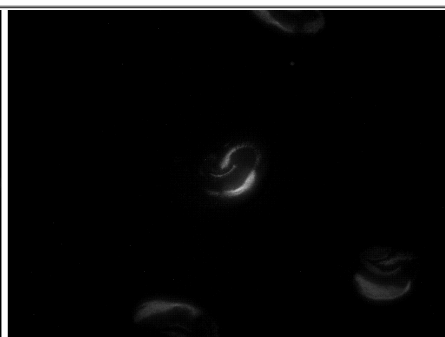
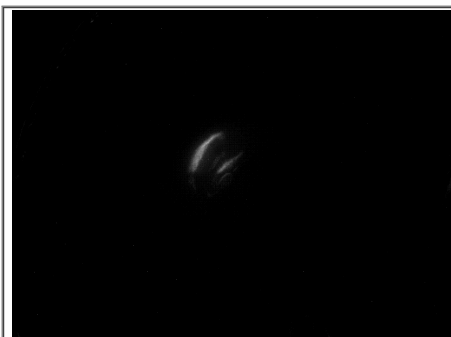
A1 NMDA 10µM

A2 NMDA 10µM +

A3 NMDA 10µM +

000007 10µM+417023 10µM

000007 10µM+417023 10µM



B1 NMDA 10µM

B2 NMDA 10µM +

B3 NMDA 10µM +

000007 100µM+417023 100µM

000007 100µM+417023 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: 7 U Screen ID: 2

Solvent Code: DMSO Solvent Prep:

Test Date: 26-May-2010

Reference: 450:185

Summary of NP Assay: Kainic acid

- Test Result: No Neuroprotection
- ADD compounds evaluated: 7 417023

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: 000007

Batch: U

Date Started: 26-May-2010

Compound 2 : ADD Number: 417023

Batch: U

Date Completed: 28-May-2010

References: 450: 185

Excitotoxin: Kainic Acid

Insult Duration: 4 Hours

Solvent: DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

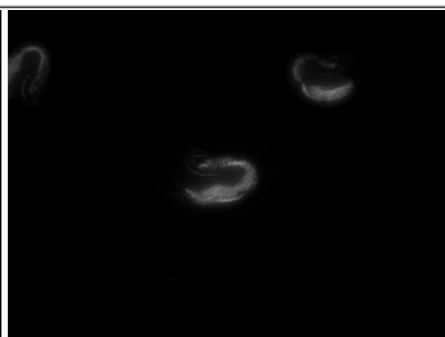
A1 KA 20µM

A2 KA 20µM +

A3 KA 20µM +

000007 10µM+417023 10µM

000007 10µM+417023 10µM



B1 KA 20µM

B2 KA 20µM +

B3 KA 20µM +

000007 100µM+417023 100µM

000007 100µM+417023 100µM

PRIMARY SCREEN EXPERIMENT DESCRIPTION

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Anticonvulsant Screening Program

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

ASP ID: 7 XX Screen ID: 3

Solvent Code: DMSO Solvent Prep:

Test Date: 27-Feb-2013

Reference: 485:210, 214

Summary of NP Assay: NMDA

● Test Result: No Neuroprotection

Comments: 7 was added 30 minutes after NMDA

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

Compound 1 : ADD Number: 000007

Batch: U

Date Started: 27-Mar-2013

Compound 2 : ADD Number:

Batch:

Date Completed: 08-Mar-2013

References: 485: 210,214

Excitotoxin: NMDA

Insult Duration: 4 Hours

Solvent: DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

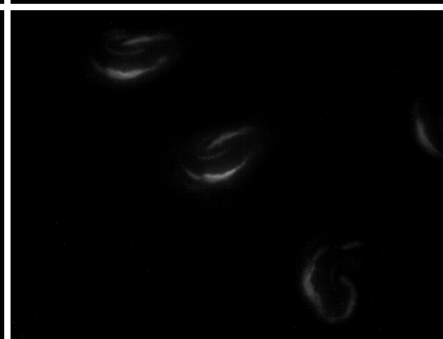
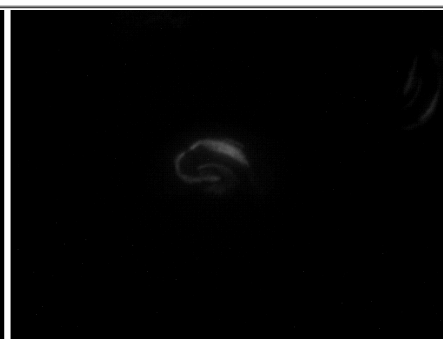
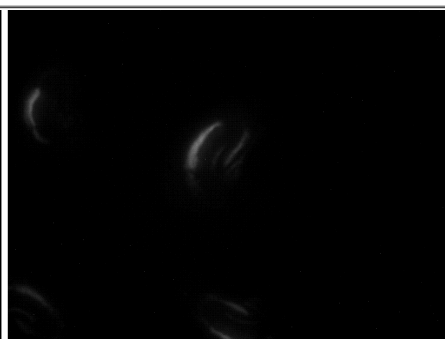
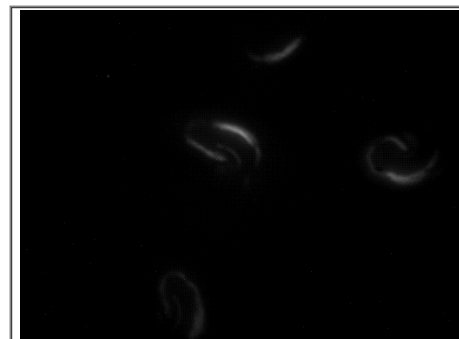
A1 NMDA 10 μ M

A2 NMDA 10 μ M +

A3 NMDA 10 μ M +

000007 10 μ M

000007 10 μ M



B1 NMDA 10 μ M

B2 NMDA 10 μ M +

B3 NMDA 10 μ M +

000007 100 μ M

000007 100 μ M

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: 7 XX Screen ID: 4

Solvent Code: DMSO Solvent Prep:

Test Date: 27-Feb-2013

Reference: 485: 210, 214

Summary of NP Assay: Kainic acid

● Test Result: No Neuroprotection

Comments: 7 was added 30 minutes after Kainic acid

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

Compound 1 : ADD Number: 000007

Batch: U

Date Started: 27-Feb-2013

Compound 2 : ADD Number:

Batch:

Date Completed: 08-Mar-2013

References: 485: 210,214

Excitotoxin: Kainic Acid

Insult Duration: 4 Hours

Solvent: DMSO

Primary Screen Results: No neuroprotection observed

EXPERIMENT IMAGES & WELL DESCRIPTION

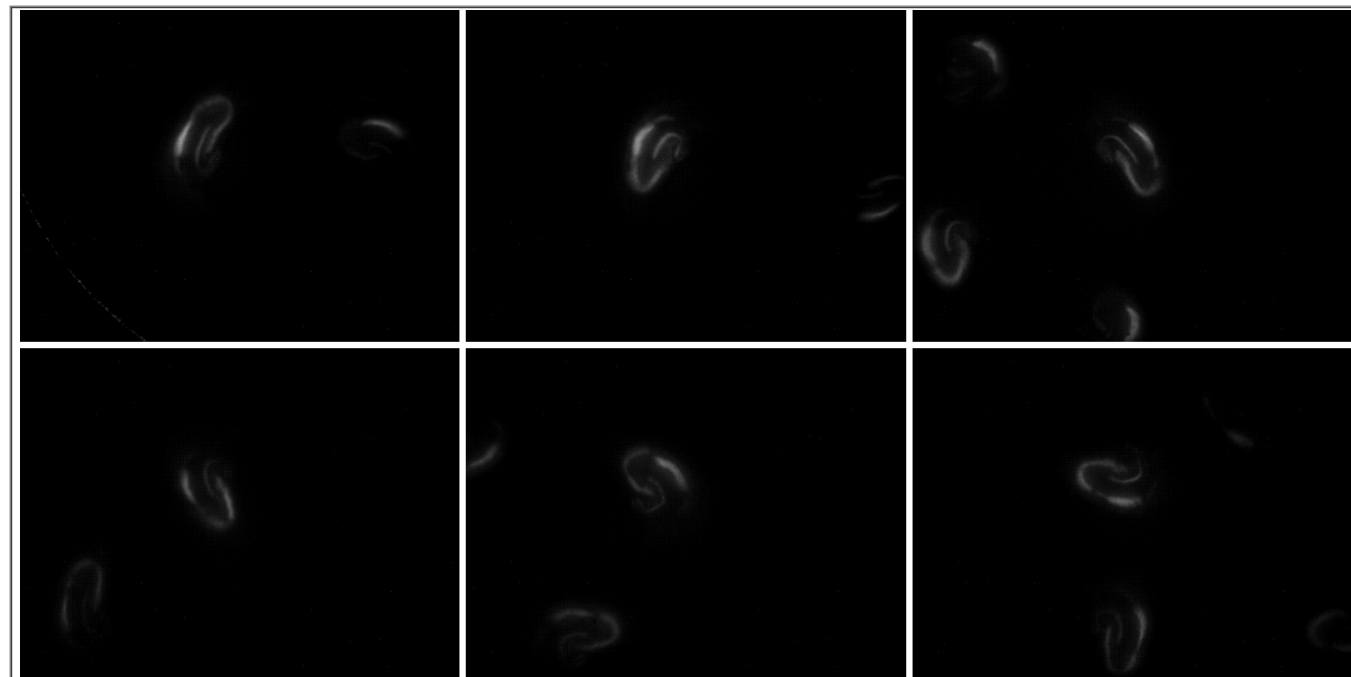
A1 KA 20µM

A2 KA 20µM +

A3 KA 20µM +

000007 10µM

000007 10µM



B1 KA 20µM

B2 KA 20µM +

B3 KA 20µM +

000007 100µM

000007 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.