# ab315371 – Human Striatal Medium Spiny GABAergic Neurons (Male, WC-30) Imaging and Plate Reader Assays

For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab315371

## Storage and Stability

Immediately transfer the vial of neurons to liquid nitrogen upon receipt. Transfer the vials of supplements to a -20°C freezer. The supplements can be stored at -20°C for up to 6 months. Alternatively, the supplements can be stored at -80°C for up to 18 months.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| One vial of 2 million cryopreserved medium spiny neurons | 500 µL | -196°C |
| BrainFast GABA Supplement (1000x) | 1 vial | -20°C or -80°C |
| BrainFast D4 Supplement (1000x) | 1 vial | -20°C or -80°C |
| BrainFast SK Supplement (1000x) | 1 vial | -20°C or -80°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- DMEM/F12 Medium (Life Technologies #11330-032)
- Neurobasal Medium (Life Technologies #21103-049)
- B27 Supplement (Life Technologies #17504-044)
- N2 Supplement (Life Technologies #17502-048)
- GlutaMAX (Life Technologies #35050-061)
- Geltrex (Life Technologies #A1413201)
- BDNF (AB217473)
- GDNF (AB259417)
- TGF-β1 (AB50036)
- PDL-Coated 96-Well Plates

## PROCEDURE

### Thawing and Seeding the Neurons

1. Gather the components for the Seeding Medium according to the recipe below. Note that BDNF, GDNF, and TGF-β1 are lyophilized powders. Follow Abcam's instructions for reconstitution. We recommend creating stock solutions of 10 µg/mL for BDNF, 10 µg/mL for GDNF, and 1 µg/mL for TGF-β1.
2. Working in a cell culture hood (biological safety cabinet), combine all components in an appropriately sized sterile container. For preparation of the Geltrex, add cold DMEM/F12 directly to an aliquot of frozen Geltrex to yield a 1:10 dilution. For example, if aliquots of Geltrex have a volume of 100 µL, add 900 µL of cold DMEM/F12. Immediately place this mixture at 4°C to allow the Geltrex to thaw and dissolve before adding the appropriate amount to the Seeding Medium. Allow the Seeding Medium to equilibrate to room temperature for at least 15 minutes. Do not warm the medium in a 37°C water bath.
3. Remove the cryovial from liquid nitrogen and place in a 37°C water bath. To minimize contamination, avoid submerging the cap. Gently move the vial within the bath to increase the rate of thawing.
4. As soon as the last of the ice melts, which will take ~75-90 seconds, remove the vial from the water bath. Disinfect the vial by spraying it with 70% ethanol and transfer it to the cell culture hood.
5. Slowly add 500 µL of seeding medium to the vial at a rate of ~1 drop/s using a 1 mL pipette tip. This process should take about 30 seconds.
6. Gently transfer all contents (~1mL total) from the vial to a new sterile 50 mL conical tube.
7. To collect any residual cells, gently add another 1 mL of seeding medium to the vial and then gently transfer to the conical tube.
8. Slowly add an additional 3 mL of seeding medium to the 50 mL conical tube using a 10 mL serological pipette. Gently swirl the conical tube while adding the medium. This process should take about 1 minute.
9. To count the cells, gently swirl the conical tube again and remove 10 µL from the cell suspension. Count the number of viable cells per mL with a hemocytometer using the trypan blue exclusion method to identify dead/viable cells.
10. The recommended seeding density is 40,000 – 50,000 viable cells/well for a 96-well plate (~125,000 – 140,000 viable cells/cm$^2$). Use the following equation to determine the volume of cell suspension needed for each 96-well plate:

    Volume of cell suspension needed (mL) = (4.8 – 5.4x10$^6$ cells)/(viable cells per mL).

11. In a separate 50 mL conical tube, add the calculated volume of cell suspension needed, and then add enough medium to obtain a final volume of 12 mL. For example, if the volume of cell suspension needed is 2 mL, combine 2 mL of cell suspension with 10 mL of medium.
12. Mix completely and then plate 100 µL/well (40,000 – 50,000 cells/well) onto a PDL-coated 96-well plate using a multi-channel pipettor or liquid handler. Throughout the seeding process, be careful not to move or agitate the plate as this may lead to uneven attachment.
13. After seeding, do not immediately transfer the plate to the incubator. Leave it in the hood for 15 minutes to allow the cells to settle to the bottom of the well. After 15 minutes, very gently transfer the plate to a humidified incubator at 37°C with 5% $CO_2$. Day of cell plating is designated as Day 0.

***Δ Note:*** *Entire thawing and plating process should not exceed 2 hours; post-thaw viability and overall cell health will be severely impacted and lead to an unsuccessful culture.*

### Day 4 Medium Addition

1. On Day 4 (96 hours after seeding), prepare fresh Day 4 Medium (see recipe below).
2. Gently add 100 µL/well to the entire plate for a total of 200 µL/well.

### Day 7 and Onward Medium Changes

1. Change half the medium (100 µL/well) twice weekly (on Day 7, 11, 14, 18, etc.) using Maintenance Medium (see recipe below).
2. The neurons mature rapidly and can be maintained viable and adherent in culture under the above conditions for at least 3 weeks post-seeding.

## Media Compositions

### Seeding Medium

| | Component | Stock Conc. | Final Conc. | 1 plate volumes | 2 plate volumes | 5 plate volumes |
|---|---|---|---|---|---|---|
| 1 | DMEM/F12 Medium | 1X | 0.5X | 9.5 mL | 19 mL | 47.5 mL |
| 2 | Neurobasal Medium | 1X | 0.5X | 9.5 mL | 19 mL | 47.5 mL |
| 3 | B27 Supplement | 50X | 1X | 400 µL | 800 µL | 2 mL |
| 4 | N2 Supplement | 100X | 1X | 200 µL | 400 µL | 1 mL |
| 5 | GlutaMAX | 200 mM | 0.5 mM | 50 µL | 100 µL | 250 µL |
| 6 | BDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 7 | GDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 8 | TGF-β1 | 1 µg/mL | 1 ng/mL | 20 µL | 40 µL | 100 µL |
| 9 | Geltrex | 15 mg/mL | 15 µg/mL | 200 µL (of 1:10) | 400 µL (of 1:10) | 1 mL (of 1:10) |
| 10 | BrainFast GABA Supplement (1000x) | 1000X | 1X | 20 µL | 40 µL | 100 µL |
| 11 | BrainFast SK Supplement (1000x) | 1000X | 1X | 20 µL | 40 µL | 100 µL |

### Day 4 Medium

| | Component | Stock Conc. | Final Conc. | 1 plate volumes | 2 plate volumes | 5 plate volumes |
|---|---|---|---|---|---|---|
| 1 | DMEM/F12 Medium | 1X | 0.5X | 9.6 mL | 19.2 mL | 48 mL |
| 2 | Neurobasal Medium | 1X | 0.5X | 9.6 mL | 19.2 mL | 48 mL |
| 3 | B27 Supplement | 50X | 1X | 400 µL | 800 µL | 2 mL |
| 4 | N2 Supplement | 100X | 1X | 200 µL | 400 µL | 1 mL |
| 5 | GlutaMAX | 200 mM | 0.5 mM | 50 µL | 100 µL | 250 µL |
| 6 | BDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 7 | GDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 8 | TGF-β1 | 1 µg/mL | 1 ng/mL | 20 µL | 40 µL | 100 µL |
| 9 | BrainFast D4 Supplement (1000x) | 1000X | 1X | 20 µL | 40 µL | 100 µL |
| 10 | BrainFast SK Supplement (1000x) | 1000X | 1X | 20 µL | 40 µL | 100 µL |

### Maintenance Medium

| | Component | Stock Conc. | Final Conc. | 1 plate volumes | 2 plate volumes | 5 plate volumes |
|---|---|---|---|---|---|---|
| 1 | DMEM/F12 Medium | 1X | 0.5X | 9.6 mL | 19.2 mL | 48 mL |
| 2 | Neurobasal Medium | 1X | 0.5X | 9.6 mL | 19.2 mL | 48 mL |
| 3 | B27 Supplement | 50X | 1X | 400 µL | 800 µL | 2 mL |
| 4 | N2 Supplement | 100X | 1X | 200 µL | 400 µL | 1 mL |
| 5 | GlutaMAX | 200 mM | 0.5 mM | 50 µL | 100 µL | 250 µL |
| 6 | BDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 7 | GDNF | 10 µg/mL | 10 ng/mL | 20 µL | 40 µL | 100 µL |
| 8 | TGF-β1 | 1 µg/mL | 1 ng/mL | 20 µL | 40 µL | 100 µL |

## Technical Support


For all technical or commercial enquiries please go to:

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