

Materials List for

A Technique to Assess the Inhibitory Effect of Toxin Exposure on Miniature Excitatory Postsynaptic Currents (mEPSCs)

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URL: <https://www.jove.com/video/22071>

DOI: [doi:10.3791/22071](https://doi.org/10.3791/22071)

Materials

| Name | Company | Catalog Number | Comments |
|-----------------------------|-------------------|----------------|---|
| Table 1. ESC and ESN | | | |
| Knockout DMEM | Life Technologies | 10829-018 | Media: ESC culture medium Formulation and notes: 500 mL |
| 100x MEM NEAA | Life Technologies | 11140-050 | Media: store at 4 °C for up to 1 month Formulation and notes: 6 mL |
| 200 mM L-Alanyl-L-Glutamine | ATCC | 30-2115 | 6 mL |
| ES qualified FBS | Applied Stem Cell | ASM-5007 | 90 mL |
| 100x antibiotics | Sigma-Aldrich | A5955 | 3 mL |
| 55 mM 2-mercaptoethanol | Life Technologies | 21985-023 | 1.1 mL |
| 107 Units/mL LIF | Millipore | ESG1107 | 60 µL |
| Knockout DMEM | Life Technologies | 10829-018 | Media: ESC differentiation medium Formulation and notes: 436.6 mL |
| 100x MEM NEAA | Life Technologies | 11140-050 | Media: store at 4 °C for up to 1 month Formulation and notes: 5 mL |
| 200 mM L-Alanyl-L-Glutamine | ATCC | 30-2115 | 5 mL |
| ESC-qualified serum | Applied Stem Cell | ASM-5007 | 50 mL |
| 100x antibiotics | Sigma-Aldrich | A5955 | 2.5 mL |
| 55 mM 2-mercaptoethanol | Life Technologies | 21985-023 | 0.9 mL |
| Dulbecco's PBS | Sigma-Aldrich | D8537 | Media: NPC trypsinization medium Formulation and notes: 100 mL |
| 0.5 M EDTA (18.3%) | Sigma-Aldrich | 3690 | Media: freeze in 5 mL aliquote Formulation and notes: 0.266 mL |
| Trypsin | Sigma-Aldrich | T8802 | 50 mg |
| Polyethyleneimine (PEI) | Sigma-Aldrich | P3143 | Media: Surface coating solutions Formulation and notes: 2.5 µg/mL in H2O |
| Poly-D-lysine (PDL) | Sigma-Aldrich | P7280 | Media: use within 1 wk Formulation and notes: 100 µg/mL in H2O |
| Laminin | Sigma-Aldrich | L2020 | 5 µg/mL in H2O |
| DMEM/F12 + GlutaMAX | Life Technologies | 10565-018 | Media: NPC culture medium Formulation and notes: 492.5 mL |
| 100x N2 vitamins | Life Technologies | 17502-048 | Media: store at 4 °C for up to 1 month Formulation and notes: 5 mL |

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| 100x antibiotics | Sigma-Aldrich | A5955 | 2.5 mL |
| Neurobasal A | Life Technologies | 10888-022 | Media: ESN culture medium |
| | | | Formulation and notes: 482.5 mL |
| 50x B27 vitamins | Life Technologies | 17504-044 | Media: store at 4 °C for up 1 month |
| | | | Formulation and notes: 10 mL |
| 200 mM L-Alanyl-L-Glutamine | ATCC | 30-2115 | 5 mL |
| 100x antibiotics | Sigma-Aldrich | A5955 | 2.5 mL |
| | | | Formulation and notes: dd 150 mg 5FDU and 350 mg uridine to 10 mL Neurobasal A. Sterile filter, aliquot and freeze. Use 2000x in ESN culture medium. |
| Uridine | Sigma-Aldrich | U3003 | |
| TrypLE Express Trypsin | Life Technologies | 12605-010 | Media: Miscellaneous |
| | | | Formulation and notes: store at RT |
| DMSO | Sigma-Aldrich | D8418 | store at RT |
| Soybean trypsin inhibitor (STI) | Sigma-Aldrich | T6414 | store at -20 °C |
| Ethanol | Sigma-Aldrich | E7023 | store at RT |
| Ascorbic acid | Sigma-Aldrich | A4403 | Prepare 100 mM stock by dissolving 100 mg ascorbic acid in 5.7 mL of 50:50 DMSO/ethanol mix. |
| Retinoic acid (RA) | Sigma-Aldrich | R2625 | Resuspend 15 mg RA in 9 mL 50:50 DMSO/ethanol. Supplement with 1 mL of 100 mM ascorbic acid stock. Aliquot and stored at -80 °C. Stable for 6 mos. |
| Table 2. MIST | | | |
| NaCl | Sigma-Aldrich | 71386 | Media: Extracellular Recording Buffer |
| | | | Final Concentration (mM): 58.44 |
| | | | MW: 140 |
| KCl | Sigma-Aldrich | 60135 | Media: (ERB; pH = 7.3, 315 mO) |
| | | | Final Concentration (mM): 74.56 |
| | | | MW: 3.5 |
| NaH ₂ PO ₄ | Sigma-Aldrich | S3139 | Media: Stable at 4 °C for at least 6 mo. |
| | | | Final Concentration (mM): 119.98 |
| | | | MW: 1.25 |
| CaCl ₂ | Sigma-Aldrich | 21115 | Final Concentration (mM): 110.98 |
| | | | MW: 2 |
| MgCl ₂ | Sigma-Aldrich | 63069 | Final Concentration (mM): 95.21 |
| | | | MW: 1 |
| D-glucose | Sigma-Aldrich | G8644 | Final Concentration (mM): 180.16 |
| | | | MW: 10 |
| HEPES | Sigma-Aldrich | H0887 | Final Concentration (mM): 238.3 |
| | | | MW: 10 |
| K-gluconate | Sigma-Aldrich | P1847 | Media: Intracellular Recording Buffer |
| | | | Final Concentration (mM): 234.5 |

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| | | | MW: 140 |
| NaCl | Sigma-Aldrich | 71386 | Media: (IRB; pH = 7.3, 320 mO) |
| | | | Final Concentration (mM): 58.44 |
| | | | MW: 5 |
| Mg-ATP | Sigma-Aldrich | A9187 | Media: Stable at 4 °C for at least 6 mo. |
| | | | Final Concentration (mM): 507.18 |
| | | | MW: 2 |
| Li-GTP | Sigma-Aldrich | G5884 | Final Concentration (mM): 523.18 |
| | | | MW: 0.5 |
| CaCl ₂ | Sigma-Aldrich | 21115 | Media: Stable at 4 °C for at least 6 mo. |
| | | | Final Concentration (mM): 110.98 |
| | | | MW: 0.1 |
| MgCl ₂ | Sigma-Aldrich | 63069 | Media: Stable at 4 °C for at least 6 mo. |
| | | | Final Concentration (mM): 95.21 |
| | | | MW: 1 |
| EGTA | Sigma-Aldrich | E3889 | 380.35 Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 380.35 MW: 1 |
| HEPES | Sigma-Aldrich | H0887 | Media: Stable at 4 °C for at least 6 mo. |
| | | | Final Concentration (mM): 238.3 |
| | | | MW: 10 |
| Bicuculline | Tocris | 131 | Media: Miscellaneous |
| | | | Final Concentration (mM): 0.01 |
| | | | MW: 417.85 |
| CNQX | Sigma-Aldrich | C239 | Final Concentration (mM): 0.01 |
| | | | MW: 276.12 |
| Tetrodotoxin | Sigma-Aldrich | A8001 | Final Concentration (mM): 0.005 |
| | | | MW: 645.74 |
| BoNT/A-/G | Metabionics | N/A | Media: |
| | | | Final Concentration (mM): TBD |
| | | | MW: 150,000 |
| Tetanus toxin | Sigma-Aldrich | T3194 | Final Concentration (mM): TBD |
| | | | MW: 150,000 |
| Sigmacote | Sigma-Aldrich | SL-2 | Final Concentration (mM): N/A |
| | | | MW: N/A |
| Table 3. Equipment and Software | | | |
| MiniAnalysis (version 6.0.7) | Synaptosoft, Inc. | N/A | Event detection software |
| Igor Pro (version 6.22A) | WaveMetrics | N/A | Software for visualization of recordings |
| Patchmaster | Heka | N/A | Data acquisition software |
| Olympus IX51 inverted microscope | Olympus | N/A | |
| Micromanipulator | Sutter Instrument | MPC-365 | |
| Patch-clamp amplifier | Heka | ECB10USB | |

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| Micropipette puller | Sutter Instrument | P-1000 | |
| Borosilicate capillary tubes | Sutter Instrument | B150-86-10 | Corning 7740 |
| 18 mm glass coverslips | Fisher | 12-545-84 | |
| Plasma cleaner | Harrick Plasma | PDC-32G | |
| Cell strainer (40 μ m) | Fisher | 08-771-1 | |
| Stovall Belly Dancer Shaker | Fisher | 15-453-211 | |
| Low adhesion dishes | Fisher | 05-539-101 | Corning 3262 |
| Bacterial dishes | VWR | 25384-302 | 100 x 15 mm |