iMPS Platedown Procedure

Protocol Number: CSMNC-SOP-C-001 - APPENDIX A

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| Media Formulations for Derivation, Propagation and Maturation of Motor Neural Cultures | | | | | | | iately before use | Light Sensitive | |
|--|------------------------|-------|------------------|-------|--------------------|------------------|-------------------|-----------------|--------------|
| Neural Differentiation Media (NDM) | Stock Concentration | Units | 1X Concentration | Units | Volume 1,000 mL | Volume 500 mL | Volume 250 | Volume 100 | Volume 50 |
| IMDM | 1X | mL | 47.5% | mL | 475 mL | 237.5 mL | 118.75 | 47.5 | 23.75 |
| F-12 | 1X | mL | 47.5% | mL | 475 mL | 237.5 mL | 118.75 | 47.5 | 23.75 |
| Non-Essential Amino Acids (NEAA) | 100X | mL | 1.0% | mL | 10 mL | 5 mL | 2.5 | 1 | 0.5 |
| B-27 | 50X | mL | 2.0% | mL | 20 mL | 10 mL | 5 | 2 | 1 |
| N-2 | 100X | mL | 1.0% | mL | 10 mL | 5 mL | 2.5 | 1 | 0.5 |
| PSA | 100X | mL | 1.0% | mL | 10 mL | 5 mL | 2.5 | 1 | 0.5 |

| Motor Neuron Maturation Media (MNMM) Stage 1 | Stock Concentration | Units | 1X Concentration | Units | Volume 500 | Volume 250 | Volume 100 | Volume 50 | Volume 25 | Volume 10 |
|--|------------------------|-------|------------------|-------|---------------|---------------|---------------|--------------|--------------|--------------|
| NDM | 1X | mL | 99.8% | mL | 498 mL | 249 mL | 99.6 mL | 49.8 | 24.9 | 9.96 |
| All-trans Retinoic Acid (ATRA) | 10 | mM | 10.0% | μМ | 5 μL | 2.5 μL | 1 μL | 0.5 | 0.25 | 0.1 |
| PMN | 10 | mM | 100.0% | μМ | 50 μL | 25 μL | 10 μL | 5 | 2.5 | 1 |
| BDNF | 10 | μg/mL | 10 | ng/mL | 500 μL | 250 μL | 100 μL | 50 | 25 | 10 |
| GDNF | 10 | μg/mL | 10 | ng/mL | 500 μL | 250 μL | 100 μL | 50 | 25 | 10 |
| Ascorbic Acid | 500 | μg/mL | 200 | ng/mL | 200 μL | 100 μL | 40 μL | 20 | 10 | 4 |
| db-cAMP | 102 | mM | 1 | μМ | 5 μL | 2.5 μL | 1 μL | 0.5 | 0.25 | 0.1 |
| DAPT | 20 | mM | 2.5 | μМ | 62.5 μL | 31.3 μL | 12.5 | 6.3 | 3.1 | 1.6 |

| Motor Neuron Maturation Media (MNMM) Stage 2 | Stock Concentration | Units | 1X Concentration | Units | Volume 500 | Volume 250 | Volume 100 | Volume 50 | Volume 25 | Volume 10 |
|--|------------------------|-------|------------------|-------|---------------|---------------|---------------|--------------|--------------|--------------|
| Neuralbasal | 1X | mL | 98.8% | mL | 482 mL | 241 mL | 96.4 mL | 48.2 | 24.1 | 9.64 |
| NEAA | 100X | mL | 1.0% | mL | 5 mL | 2.5 mL | 1 mL | 0.5 mL | 0.25 mL | 0.1 mL |
| Glutamax | 200X | mL | 0.5% | mL | 2.5 mL | 1.25 mL | 0.5 mL | 0.25 | 0.125 | 0.05 |
| N-2 | 100X | mL | 1.0% | mL | 5 mL | 2.5 mL | 1 mL | 0.5 | 0.25 | 0.1 |
| BDNF | 10 | μg/mL | 10 | ng/mL | 500 μL | 250 μL | 100 μL | 50 | 25 | 10 |
| GDNF | 10 | μg/mL | 10 | ng/mL | 500 μL | 250 μL | 100 μL | 50 | 25 | 10 |
| Ascorbic Acid | 500 | μg/mL | 200 | ng/mL | 200 μL | 100 μL | 40 μL | 20 | 10 | 4 |
| db-cAMP | 102 | mM | 1 | μМ | 5 μL | 2.5 μL | 1 μL | 0.5 | 0.25 | 0.1 |
| Ara-C | 10 | mM | 0 | μМ | 5 μL | 2.5 μL | 1 μL | 0.5 | 0.25 | 0.1 |
| PSA | 100X | mL | 0.01 | mL | 5 mL | 2.5 mL | 1 mL | 0.5 | 0.25 | 0.1 |