

Materials List for:

# Derivation of Enriched Oligodendrocyte Cultures and Oligodendrocyte/Neuron Myelinating Co-cultures from Post-natal Murine Tissues

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## Materials

| Name   | Company                     | Catalog Number | Comments |
|--|-----------------------------|----------------|----------|
| Dulbecco's Modified Eagle Medium (DMEM)            | Multicell                   | 319-005-CL     |          |
| Hank's Balanced Salt Solution (HBSS)               | Invitrogen                  | 14170-112      |          |
| Minimum Essential Media (MEM)                      | GIBCO, by Life Technologies | 12360-038      |          |
| Fetal Bovine Serum (FBS)                           | GIBCO, by Life Technologies | 10091-148      |          |
| Penicillin-Streptomycin (Pen/Strep)                | GIBCO, by Life Technologies | 15140-122      |          |
| GlutaMAX   | Invitrogen                  | 35050-061      |          |
| Poly-L-lysine                                      | Sigma-Aldrich               | P2636          |          |
| Bovine Serum Albumin (BSA)                         | Sigma-Aldrich               | A4503          |          |
| Human merosin purified protein (LN2)               | EMD Millipore               | CC085          |          |
| Recombinant rat ciliary neurotrophic factor (CNTF) | PeptoTech Inc               | 450-50         |          |
| L-thyroxine  | Biochemika                  | 89430          |          |
| Holo-transferrin                                   | Sigma-Aldrich               | T0665          |          |
| B27 supplement                                     | GIBCO, by Life Technologies | 0080085-SA     |          |
| Bovine insulin                                     | Sigma-Aldrich               | I6634          |          |
| 3,3',5-Triiodo-L-thyronine                         | Sigma-Aldrich               | I6634          |          |
| Progesterone                                       | Sigma-Aldrich               | P8783          |          |
| Putrescine   | Sigma-Aldrich               | P7505          |          |
| Sodium Selenite                                    | Sigma-Aldrich               | S5261          |          |
| 5-Fluoro-2'-deoxyuridine (FuDR)                    | Sigma-Aldrich               | F0503          |          |
| Papain solution                                    | Worthington Biochemical     | LS003126       |          |
| DNaseI   | Roche Group                 | 1010159001     |          |
| L-cysteine   | Sigma-Aldrich               | C7352          |          |
| 24-well tissue culture dishes                      | Cellstar                    | 662-160        |          |
| T25-tissue culture flasks with vent cap            | Corning                     | 430639         |          |
| 10 cm tissue culture dishes                        | Corning                     | 430167         |          |
| 10 cm petri dish                                   | Fisher Scientific           | 0875713        |          |
| Collagenase A                                      | Roche Group                 | 103578         |          |
| CellTrics 50 µm filter (optional)                  | PARTEC                      | 04-004-2327    |          |

|   |                                |             |  |
|---|--------------------------------|-------------|--|
| Myelin Basic Protein (MBP) Antibody                           | AbD Serotec                    | MCA409S     |  |
| NG2 antibody  | EMD Millipore                  | AB5320      |  |
| Myelin-Associated Glycoprotein (MAG) antibody                 | EMD Millipore                  | MAB1567     |  |
| 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP) antibody   | Covance                        | SMI-91R-100 |  |
| Actin pan Ab-5 antibody                                       | Fitzgerald                     | 10R-A106AX  |  |
| Neurofilament-200 (NF) antibody                               | Sigma-Aldrich                  | N4142       |  |
| Tubulin beta-3 chain (Tuj1) antibody                          | EMD Millipore                  | MAB5544     |  |
| Alexa Fluor 488 goat anti-rabbit IgG (H+L) secondary antibody | Invitrogen                     | A11008      |  |
| Alexa Fluor 555 goat anti-mouse IgG (H+L) secondary antibody  | Invitrogen                     | A21422      |  |
| Alexa Fluor 647 goat anti-rat (IgG) (H+L) secondary antibody  | Invitrogen                     | A21247      |  |
| Goat anti-mouse IgG (H+L)-HRP conjugated secondary antibody   | Bio-Rad                        | 170-6516    |  |
| Goat anti-rat IgG (H+L)-HRP conjugated secondary antibody     | Santa Cruz Biotechnology, Inc. | SC-2065     |  |
| 4',6-diamidino-2-phenylindole (DAPI)                          | Sigma-Aldrich                  | D9542       |  |

**Media Recipes**

**100X OL-Supplement\***

| Ingredient                 | Amount to add |
|----------------------------|---------------|
| DMEM                       | 100 mL        |
| BSA                        | 1.02 g        |
| Progesterone               | 0.6 mg        |
| Putrescine                 | 161 mg        |
| Sodium Selenite            | 0.05 mg       |
| 3,3',5-Triiodo-L-thyronine | 4 mg          |

\*Store at -80 °C in 250 µL aliquots

**OL media**

| Ingredient                             | Amount to add |
|--|---------------|
| DMEM                                   | 23.75 mL      |
| 100X OL-Supplement                     | 250 µL        |
| Bovine insulin (from 1 mg/mL stock)    | 125 µL        |
| GlutaMAX                               | 250 µL        |
| Holo-transferrin (from 33 mg/mL stock) | 37.5 µL       |
| B27 Supplement                         | 500 µL        |
| FBS                                    | 125 µL        |
| CNTF (from 50 ng/µL stock)             | 25 µL         |

**Mixed glial culture media (made up in DMEM)**

| Ingredient                   | Final concentration                              |
|------------------------------|--|
| FBS                          | 10%  |
| Pen/Strep (0.33% from stock) | 33 units/mL Penicillin and 33 µg/mL Streptomycin |

|   |  |
|---|--|
| GlutaMAX  | 1%   |
| <b>DRGN media (made up in DMEM)</b>                 |  |
| <b>Ingredient</b>                                   | <b>Final concentration</b>                         |
| FBS   | 10%  |
| Pen/Strep (1% from stock)                           | 100 units/mL Penicillin and 100 µg/mL Streptomycin |
| <b>Digestion Solution Recipes:</b>                  |  |
| <b>OPC papain solution (made up in MEM)</b>         |  |
| <b>Ingredient</b>                                   | <b>Final concentration</b>                         |
| Papain solution                                     | 1.54 mg/mL   |
| L-cysteine  | 360 µg/mL  |
| DNaseI  | 60 µg/mL   |
| <b>DRG papain solution (made up in HBSS)</b>        |  |
| <b>Ingredient</b>                                   | <b>Final concentration</b>                         |
| Papain  | 1.54 mg/mL   |
| L-cysteine  | 360 µg/mL  |
| <b>DRG Collagenase A solution (made up in HBSS)</b> |  |
| <b>Ingredient</b>                                   | <b>Final concentration</b>                         |
| Collagenase A                                       | 4 mg/mL  |