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|  | SOP-BCR-4.25 | Preparation of Methyl Cellulose 10X for mammosphere assays | Author: S. Clouthier <i>S. Clouthier</i> | Rev: 1.0 | Issued: 4/21/10 |
| | | | Approved: M. Wicha <i>M. Wicha</i> | | Revised: 7/17/12 |

1.0 Purpose

SOP 4.25 details the preparation of methyl cellulose 10X media for mammosphere assays.

2.0 Scope

SOP 4.25 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

Reagents for 10X Growth Factor (GF) + Supplements:

| No. | Name | Amount | Storage Location |
|-----|-------------------------------------|---------|--|
| 1.0 | B27 (50X stock) | 6 ml | Freezer #2 (026-328S-A) |
| 2.0 | Antibiotic-Antimicotic (100X stock) | 3 ml | Freezer #2 (026-328S-A) |
| 3.0 | Gentamycin 50ug/ml | 24 µL | Antibiotic box- Fridge #1 (026-328S-A) |
| 4.0 | Hydrocortisone (2 mg/mL stock) | 300 µL | Freezer #2 (026-328S-A) |
| 5.0 | Insulin (1mg/ml) | 1.5mL | Freezer #2 (026-328S-A) |
| 6.0 | EGF 10ng/µ | 600 µL | Freezer #2 (026-328S-A) |
| 7.0 | bFGF 20ng/µ | 300 µL | Freezer #2 (026-328S-A) |
| 8.0 | Beta-mercaptoethanol (1:5000) | 60 µL | Fridge #4 (026-314S-A) bottom shelf |
| 8.0 | Media | 18.4 mL | Freezer #2 (026-328S-A) |
| | | | Total Volume: approx 30 mL |

Constitute Methyl Cellulose (MC):

| No. | Name | Amount |
|------------------|---------|--------|
| For 3 mL: | | |
| 1.0 | 10X GF | 0.3mL |
| 2.0 | 2.6% MC | 0.3mL |
| 3.0 | Media | 1 mL |
| 4.0 | Cells | 1.4 mL |

| No. | Name | Amount |
|-------------------|---------|--------|
| For 30 mL: | | |
| 1.0 | 10X GF | 3 mL |
| 2.0 | 2.6% MC | 3 mL |
| 3.0 | Media | 10 mL |
| 4.0 | Cells | 14 mL |

4.0 Procedure

4.1 Mix all ingredients together.

5.0 Applicable References

- 5.1 SOP 4.6: Preparation of Insulin
- 5.2 SOP 4.13: Preparation of EGF
- 5.3 SOP 4.11: Preparation of bFGF
- 5.4 SOP 4.5: Preparation of HC

6.0 Change Description

| Revision | Date | Reference | Description of Change |
|----------|---------|-----------|-----------------------|
| 1.0 | 7/17/12 | CL | Added room locations |