



WiCell Feeder Independent Pluripotent Stem Cell Protocols

Supplement: Culturing with G418

STANDARD OPERATING PROCEDURE



Feeder Independent Stem Cell Protocols Supplement: Culturing with G418

SOP Number: SOP-SH-007

Version: A

1. PURPOSE

1.1. This Standard Operating Procedure (SOP) provides additional information for culturing pluripotent stem cells with a Geneticin (G418) additive. If you have any additional questions, please contact us through technical support on the WiCell website at www.wicell.org.

2. REFERENCES

2.1. WiCell SOP-SH-002

3. DEFINITIONS (Not Applicable)

4. REQUIRED MATERIAL

4.1. Geneticin (G418: Life Technologies, 11811)

5. PROCEDURE

5.1. Thaw

Note: Before thawing, check the certificate of analysis included in the shipping packet insert to determine the recommended number thaw ratio (number of wells into which a single vials of cells should be thawed).

5.1.1. Thaw according to the WiCell Feeder Independent mTeSR1 Pluripotent Stem Cell Protocol (SOP-SH-002). G418 or any other antibiotic is not used in the thaw medium.

5.1.2. Starting with the second feed from thaw, modify medium to contain 50µg/ml Geneticin. Continue to observe cells and feed daily with mTeSR1 + 50µg/ml Geneticin until the culture requires passaging.

5.2. Passage


5.2.1. Passage according to the WiCell Feeder Independent mTeSR1 Pluripotent Stem Cell Protocols. G418 is not used on the passaging medium.

5.2.2. Beginning the day after passage, feed daily with cell culture medium (i.e. mTeSR1, E8, etc.) + 50µg/ml Geneticin according to the WiCell Feeder Independent Pluripotent Stem Cell Protocols.

5.3. Cryopreservation

5.3.1. Cryopreserve cells by removing Geneticin containing medium from the wells, and follow the WiCell Feeder Independent Pluripotent Stem Cell Protocols.

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5.4. Guide

| Activity | Medium Type |
|--------------------------------|---|
| Thaw | Cell Culture Medium |
| Feed day after thaw | Cell Culture Medium |
| Subsequent feedings after thaw | Cell Culture Medium + 50µg/ml of G-418 |
| Passage | Cell Culture Medium |
| Feedings after passage | Cell Culture Medium + 50µg/ml of G-418 |
| Cryopreservation | Cell Culture Medium |

Note: Cell Culture Medium is pluripotent stem cell culture medium (i.e. mTeSR1, E8, etc.).

4. REVISION HISTORY

| Version | Change Description | Effective Date |
|---------|-------------------------------|----------------|
| A | CC00515, Document Initiation. | See SPDC |

5. APPROVALS

| | |
|---|---|
| 7/15/2016 | 2/18/2016 |
| X JKG <hr/> | X TEL <hr/> |
| JKG Quality Assurance Manager Signed by: Gay, Jenna | TEL Director - WiCell Stem Cell Bank Signed by: Ludwig, Tenneille |