



WiCell Feeder Independent Pluripotent Stem Cell Protocols

Supplement: Culturing with Hygromycin and Zeocin

STANDARD OPERATING PROCEDURE



Title: Feeder Independent Stem Cell Protocol Supplement: Culturing with Zeocin and Hygromycin

SOP Number: SOP-SH-011

Version: B

1. PURPOSE

- 1.1. This Standard Operating Procedure (SOP) provides additional information culturing human Pluripotent Stem Cells with Zeocin and Hygromycin B additives. 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. REQUIRED MATERIAL

- 3.1. Zeocin (Invitrogen, R250-01)
- 3.2. Hygromycin B (Life Technologies 10687-010)

4. PROCEDURE

4.1. 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).

- 4.1.1. Thaw according to WiCell Feeder Independent Pluripotent Stem Cell Protocol (SOP-SH-002). Zeocin and Hygromycin B additives should not be used in the thaw medium.

- 4.1.2. On the third or fourth day after thaw, feed as per the WiCell Feeder Independent Pluripotent Stem Cell Protocol with mTeSR1+1ug/ml of Zeocin and 10ug/ml of Hygromycin B.

4.2. Culture

- 4.2.1. Continue to observe cells and feed with mTeSR1 containing Zeocin and Hygromycin B daily until cells require passaging. The cells should maintain a morphology similar to unmodified WA09 cell cultures.

4.3. Passage

- 4.3.1. Passage according to the WiCell Feeder Independent Pluripotent Stem Cell Protocol. During all passages, use medium that does not contain Zeocin and Hygromycin B.
- 4.3.2. The following day and throughout the passage, feed the cells daily with mTeSR1+1ug/ml of Zeocin and 10ug/ml of Hygromycin B. Expect to see increased cell debris from non-resistant differentiated cells killed by the antibiotic.

4.4. Cryopreservation

- 4.4.1. Cryopreserve cells by removing Zeocin and Hygromycin B containing medium from the wells, and follow the WiCell Feeder Independent Pluripotent Stem Cell Protocol.

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5. REVISION HISTORY

Version	Change Description	Effective Date
A	CC00585, Document Initiation.	4/29/2016
B	CC00690, Corrected the SOP ID from SOP-SH-008 to SOP-SH-011	See SPDC

6. APPROVALS

9/1/2017	7/12/2017
X JKG	X TEL
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JKG Quality Assurance Manager Signed by: Gay, Jenna	TEL Director of WiCell Stem Cell Bank Signed by: Ludwig, Tenneille