

STEM CELL LABORATORY (STCL)



DOCUMENT NUMBER: STCL-SOP-052 JA1

DOCUMENT TITLE:

HPC Processing of Fresh UCB Samples - Flow Chart JA1

DOCUMENT NOTES:

Document required for the BLA.

Document Information

Revision: 01 Vault: STCL-Processing-rel

Status: Release Document Type: SOPs

Date Information

Creation Date: 07 Feb 2012 Release Date: 28 Feb 2012

Effective Date: 28 Feb 2012 Expiration Date:

Control Information

Author: WATE02 Owner: WATE02

Previous Number: Change Number: STCL-CCR-010

HPCA Processing of Fresh UCB samples - Flow Chart

Sample, worksheets and labels arrive to the HPCA area and are stamped with delivery time

thoCult syringes) are

Reagent supplies (IMDM tubes and MethoCult syringes) are removed from the refrigerator; set timer for 15 minutes to allow reagents to come to room temperature

Specimen(s) is (are) logged-in by HPCA Tech and alphabet letter is assigned to UCB worksheets, sample tube, and the labels.

Specimens, information packets (worksheets), and labels are delivered to plating station (Wear PPE!)

First specimen is placed under the hood, barcode labels are placed on 0.5 and 0.4 IMDM tubes, prepared cell culture plate and specimen rack

Vortex fresh UCB at ~3000 rpm for 1-3 seconds

Obtain 2.5 $\times 10^5$ cells from the fresh UCB and dilute by adding the calculated amount of sample into the 0.5 ml IMDM tube. Rinse tip 3-5 times. Vortex to mix at ~3000 rpm for 1-3 seconds

Transfer 100 μl of the cell mixture to the tube containing 0.4ml of IMDM.

Rinse tip 1-3 times. Vortex at ~3000 rpm for 1-3 seconds

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Dispense the 1.5 of MethoCult in the syringe into the tube containing the 0.4 ml of IMDM + 100 μ l of cell mixture by allowing the MethoCult to run slowly down the side of the tube.

Mix the contents thoroughly by gently aspirating the cell mixture into the syringe and dispensing it back into the tube. REPEAT X 5

After the last mixing cycle, allow the empty syringe to sit inside the tube and allow the solution to rest for 5 minutes before plating. **Set a timer for 5 minutes.**

At this time, you may proceed with the next specimen. Each specimen must have its own rack and no more than 3 specimens will be allowed under the hood at one time.

At the end of the 5 minutes, slowly draw the MethoCult/cell mixture back up into the syringe.

Plate in triplicate. Remove the blunt-end needle from the syringe by twisting the needle using the needle cover. Remove the lid of the plate and place the cell suspension into each of the 3 wells. Dispense one drop a time into each well, beginning with the well on the left, until all of the mixture is gone.

Repeat step above with other specimens that are in-progress.

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Replace the lid on the plate. Using aseptic technique, swirl the plate to cover the bottom of each well and to remove any visible bubbles.

Upon completion of this HPC assay, deliver the remaining UCB specimen(s), labels, and information packet(s) (worksheets) to the Flow Cytometry area for testing.

Place tissue culture plate into a 37°C humidified incubator in 5% CO2 for 14-16 days

NOTE: Deliver the UCB PRE and POST slides to the microscopy area within the STCL. Slides will be read and results reported as time permits.

Signature Manifest

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All dates and times are in Eastern Time.

STCL-SOP-052 JA1 UCB HPC Flow CHart

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Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		09 Feb 2012, 12:11:20 PM	Approved

Manager Approval

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		09 Feb 2012, 12:11:43 PM	Approved

Medical Director Approval

Name/Signature		Title	Date	Meaning/Reason
Joanne Kurtzbe (KURTZ001)	rg		09 Feb 2012, 12:46:53 PM	Approved

QA Approval

Name/Signature	Title	Date	Meaning/Reason
Linda Sledge (SLEDG006)		09 Feb 2012, 07:07:01 PM	Approved

Notification

Name/Signature	Title	Date	Meaning/Reason
Linda Sledge (SLEDG006)		09 Feb 2012, 07:07:02 PM	Email Sent

Quick Approval

Approve Now

Name/Signature	Title	Date	Meaning/Reason
System Administrator (SYSADMIN)		28 Feb 2012, 12:46:49 PM	Approved

Review: STCL-SOP-052 JA1 01

Review Name/Signature Title Date Meaning/Reason Barbara Waters-Pick AVATEGO 04 Feb 2013, 05:48:10 PM Reviewed

 (WATE02)
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 Betsy Jordan (BJ42)
 06 Feb 2013, 07:44:34 AM
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 Joanne Kurtzberg (KURTZ001)
 07 Feb 2013, 10:07:54 AM
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 Linda Sledge (SLEDG006)
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Review: STCL-SOP-052 JA1 01

Review

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		10 Dec 2014, 11:06:56 PM	Reviewed
Betsy Jordan (BJ42)		11 Dec 2014, 11:13:44 AM	Reviewed
John Carpenter (JPC27)		11 Dec 2014, 12:23:57 PM	Reviewed
Joanne Kurtzberg (KURTZ001)		11 Dec 2014, 08:05:08 PM	Reviewed
Sharon Hartis (SH259)		12 Dec 2014, 09:05:56 AM	Reviewed