



STEM CELL LABORATORY (STCL)



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DOCUMENT TITLE:

Cellometer Program Used for Thawed MSC Products JA2

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Control Information

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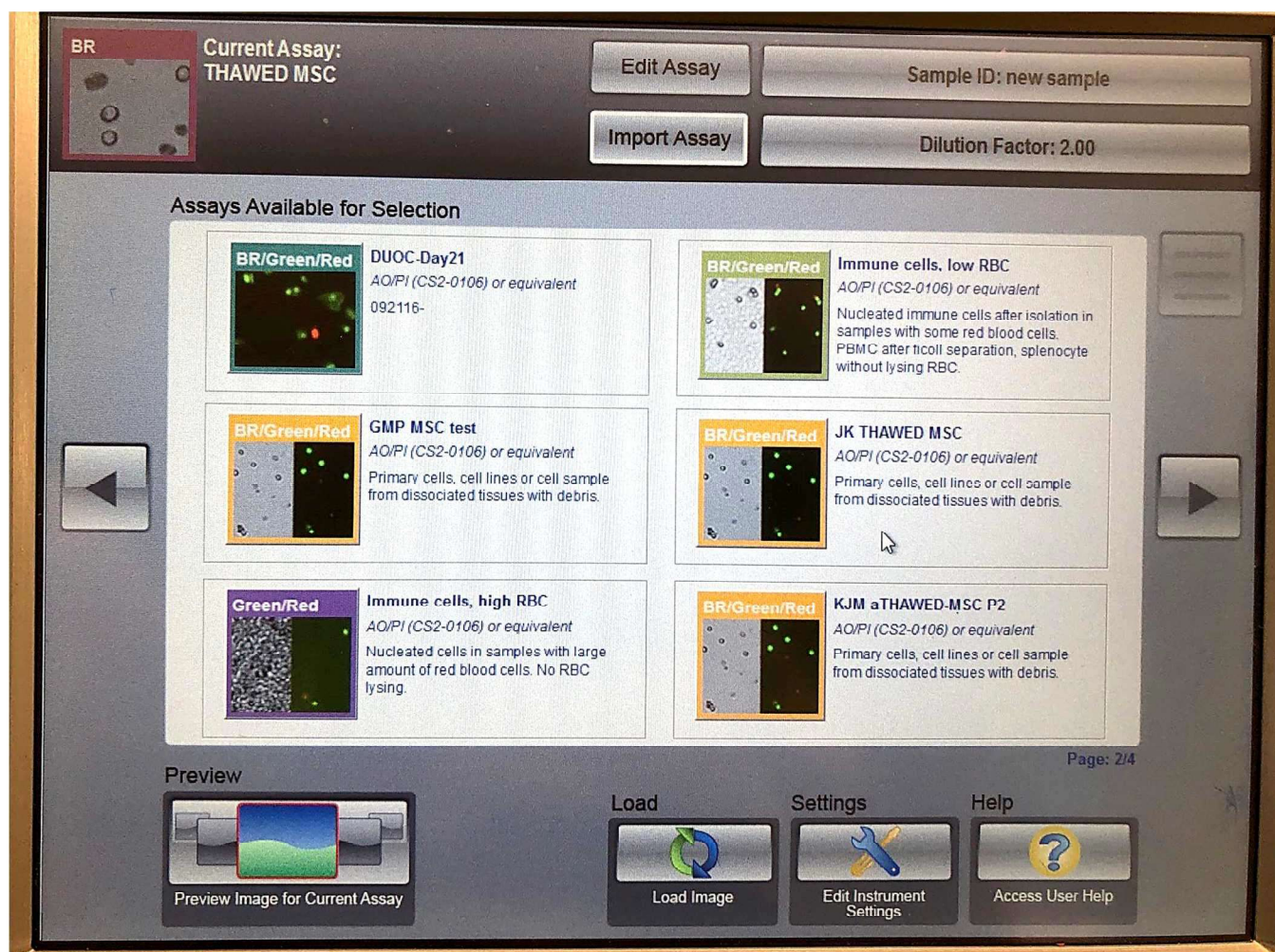
Change Number: STCL-CCR-511

STCL-EQUIP-022 (JA2) Cellometer Program Used for Thawed MSC Products


NOTE: The Cell Type most recently used for Channel 1 was Assay 2 Immune Cells Live and Cell Type most recently used for Channel 2 was Assay 2 Immune Cells Dead. These Cell Types may be used as a starting template for future assay updates; however, parameters within these Cell Types have been modified for both Channel 1 and Channel 2 as summarized below.

Checking the Assay settings: If Cell Type is locked, the settings can only be viewed. Minus a few images, this job aid show images for which **JK Thawed MSC** as an unlocked assay with unlocked Cell Types for the purpose of defining the assay parameters but also includes instructions for viewing/modifying settings in a locked Assay with locked Cell Types.

Highlight the correct assay, then click on “Edit Assay”



Assay Parameters



JK THAWED MSC
AO/PI (CS2-0106) or equivalent
 Primary cells, cell lines or cell sample
 from dissociated tissues with debris.

Unlocked for Editing

Edit

Print

Imaging Mode: Two Fluorescent Stain

Edit

Channel 1

Cell Type: Thawed MSC Ch1
 Stain: AO Live Cells
 Light Source: 470 nm
 Peak Detection: 535 nm

Edit

Channel 2

Cell Type: Thawed MSC Ch2
 Stain: PI Dead Cells
 Light Source: 540 nm
 Peak Detection: 605 nm

Edit

FL Exposure Time

900 msec

FL Exposure Time

3.0 sec

Advanced Settings

Edit

Calculation: $F1/(F1 + F2) * 100\%$
 Reports

Edit

Cancel

Save

The user may click on Print to print a summary of the settings. See the attached settings at the end of this Job Aid for the printed summary of **JK Thawed MSC** assay settings.

To modify a locked assay, it must be renamed. If an assay is locked, click on Edit next to “Locked for editing”

Assay Descriptions - Renaming a new copy to update an assay that is locked.

Select the assay title located under Assay Name, and change the name of the assay.

The prompt to “KEEP” or “CHANGE the cell type name to match the new assay name will appear if the assay title has been changed.

When prompted, click on “**CHANGE**” so that any changes made to Cell Type won’t affect other assays that share the same Cell Type. Slide the **LOCK** to off so that the Assay can be revised and click on **SAVE**.

Under Channel 1, Cell Type click on “Edit”

NOTE: For this example, Cell Type is unlocked for editing and may not be viewed when Cell Type is locked.

Assay Parameters

BR/Green/Red JK THAWED MSC
AO/PI (CS2-0106) or equivalent
Primary cells, cell lines or cell sample from dissociated tissues with debris.

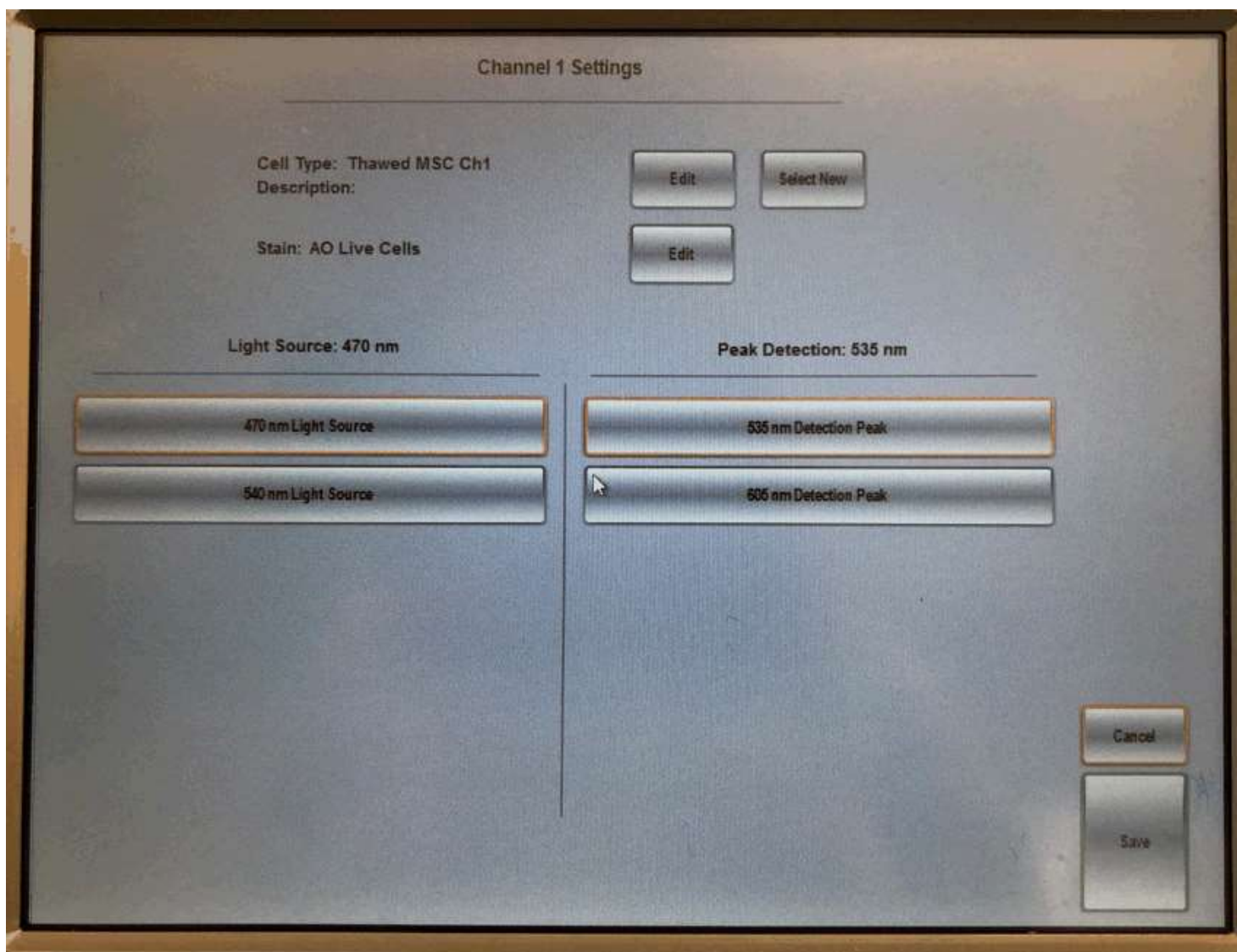
Unlocked for Editing

Edit

Print

Imaging Mode: Two Fluorescent Stain Edit

Channel 1	Channel 2
Cell Type: Thawed MSC Ch1 Stain: AO Live Cells Light Source: 470 nm Peak Detection: 535 nm Edit	Cell Type: Thawed MSC Ch2 Stain: PI Dead Cells Light Source: 540 nm Peak Detection: 605 nm Edit
FL Exposure Time: 800 msec	FL Exposure Time: 3.0 sec
Advanced Settings Edit	
Calculation: $F1/(F1 + F2) \times 100\%$ Reports Edit	Cancel
	Save



Channel 1 Settings

Confirm the Light Source and Peak Detection settings in this screen match what is pictured above and that the correct Cell Type is listed (*for this assay: **Thawed MSC Ch1***). Verify the **STAIN** is set to AO Live Cells.

If modifying an assay that has locked Cell Type, click on **SELECT NEW** to choose an unlocked Cell Type to work from. Highlight the desired Cell Type, rename the Cell Type to avoid making inadvertent changes to assays that share the same Cell Type, and click **DONE**

To the Right of Cell Type, **Thawed MSC Ch1**, click on Edit to view Cell Type parameters (this feature will only work when Cell Type is unlocked)

Cell Type Parameters

Cell Type: Thawed MSC Ch1
Description: Edit

Cell Diameter (microns) ? Min Size 5.0 Max Size 40.0

Roundness ? 0.10

Contrast Enhancement ? 0.40

Decluster ? On

Edge Factor ? 0.5

Th Factor ? 1.0

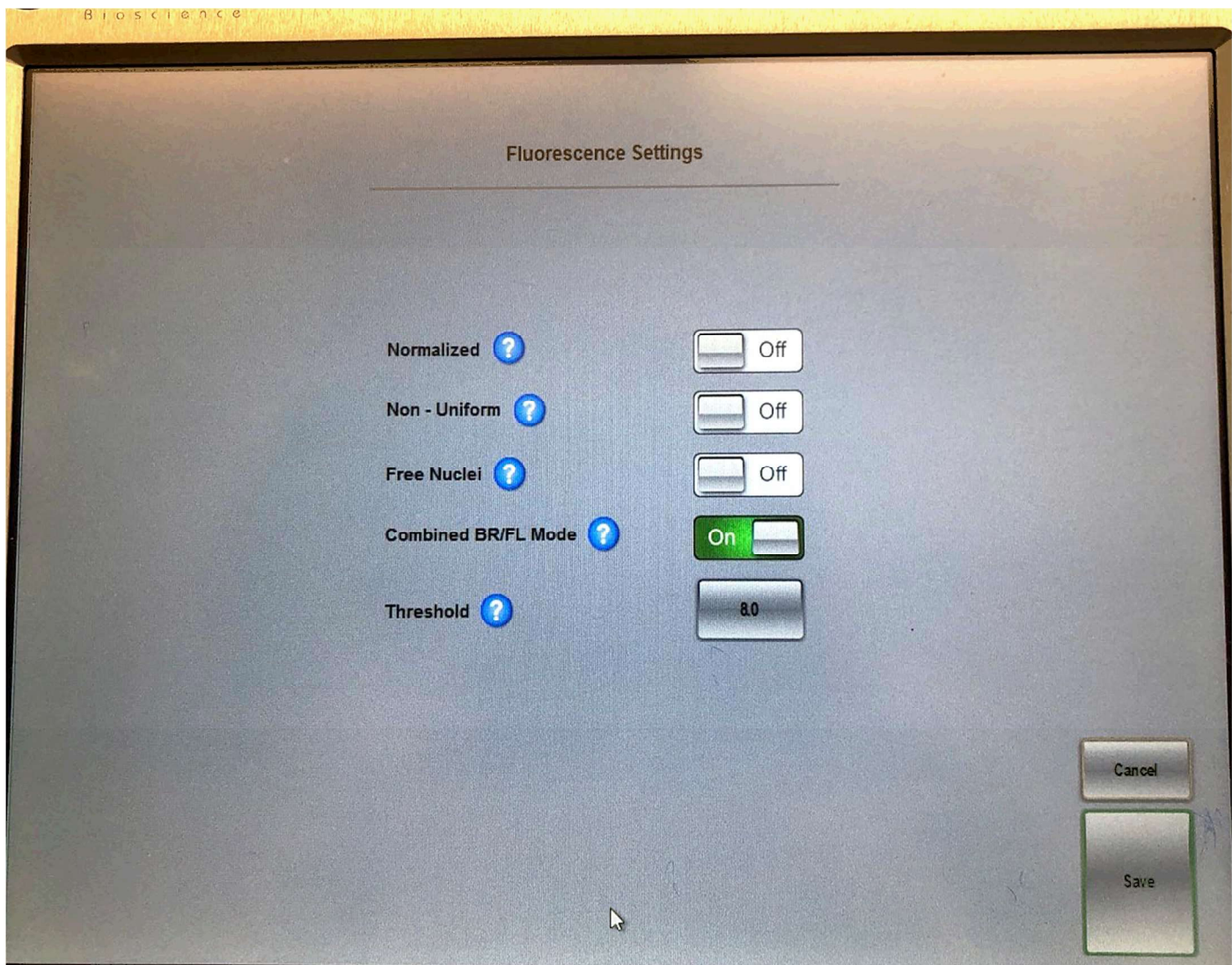
Background Adjust ? 1.0

Advanced Options Trypan FL

Print
Cancel
Save

Channel 1 Settings – Cell Type Parameters

Click on **Advanced Options** for “FL”



Channel 1 Settings –Fluorescence Settings

Confirm the settings match those listed above:

- **Threshold** should equal 8.0 **for Channel 1**
- **Combined BR/FL Mode** is ON
- Click on **SAVE** to save changes or **CANCEL** if no changes were made.

Cell Type Parameters

Cell Type: Thawed MSC Ch1 Edit

Description:

Cell Diameter (microns) ? Min Size Max Size

Roundness ?

Contrast Enhancement ?

Decluster ? On

Edge Factor ?

Th Factor ?

Background Adjust ?

Advanced Options

Channel 1 Settings – Cell Type Parameters

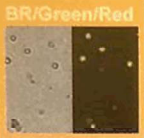
Confirm the settings match those pictured above. **Minimum cell diameter:** 5.0 and **Max Cell Diameter:** 40.0.

If modifying an unlocked Cell Type, it is recommended to change the cell type name in this window by clicking on **EDIT** to the right of Cell Type and enter a new Cell Type Name below Cell Name. To Lock the Cell type, in the same edit Cell Type window, slide the option to “**Lock Cell Type from Future Editing**” to ON. Once prompted, click on **LOCK**. Click on **SAVE**. This step will need to be completed for both Channel 1 and Channel 2 settings if locking Cell Type.

Click on Save if any changes were made, or cancel if no changes were made.

Underneath **Channel 2**, click on “**Edit**” to the right of **Cell Type**.

Assay Parameters



BR/Green/Red

THAWED MSC
AO/PI (CS2-0106) or equivalent
 Primary cells, cell lines or cell sample from dissociated tissues with debris.

Unlocked for Editing

Imaging Mode: Two Fluorescent Stain

Channel 1	Channel 2
<p>Cell Type: Thawed MSC Ch1 Stain: AO Live Cells Light Source: 470 nm Peak Detection: 535 nm</p> <p style="text-align: right;"><input type="button" value="Edit"/></p>	<p>Cell Type: Thawed MSC Ch2 Stain: PI Dead Cells Light Source: 540 nm Peak Detection: 605 nm</p> <p style="text-align: right;"><input type="button" value="Edit"/></p>
<p>FL Exposure Time</p> <p style="text-align: right;"><input type="button" value="800 msec."/></p>	<p>FL Exposure Time</p> <p style="text-align: right;"><input type="button" value="3.0 sec."/></p>

Advanced Settings

Calculation: $F1/(F1 + F2) * 100\%$
Reports

Channel 2 Settings

Cell Type: Thawed MSC Ch2
Description: Edit Select New

Stain: PI Dead Cells Edit

Light Source: 540 nm

470 nm Light Source

540 nm Light Source

Peak Detection: 605 nm

535 nm Detection Peak

605 nm Detection Peak

Cancel
Save

Channel 2 Settings

Confirm the Light Source and Peak Detection settings in this screen match what is pictured above and that the correct Cell Type is listed (for this assay: Thawed MSC Ch2). Verify the **STAIN** is set to PI Dead Cells.

If modifying an assay that has locked Cell Type, click on **SELECT NEW** to choose an unlocked Cell Type to work from. Highlight the desired Cell Type, rename the Cell Type to avoid making inadvertent changes to assays that share the same Cell Type, and click **DONE**

To the Right of Cell Type, **Thawed MSC Ch2**, click on Edit to view Cell Type parameters (*this feature will only work when Cell Type is unlocked*)

Cell Type Parameters

Cell Type: Thawed MSC Ch2

Description:

Cell Diameter (microns) ?

Roundness ?

Contrast Enhancement ?

Decluster ?

Edge Factor ?

Th Factor ?

Background Adjust ?

Min Size

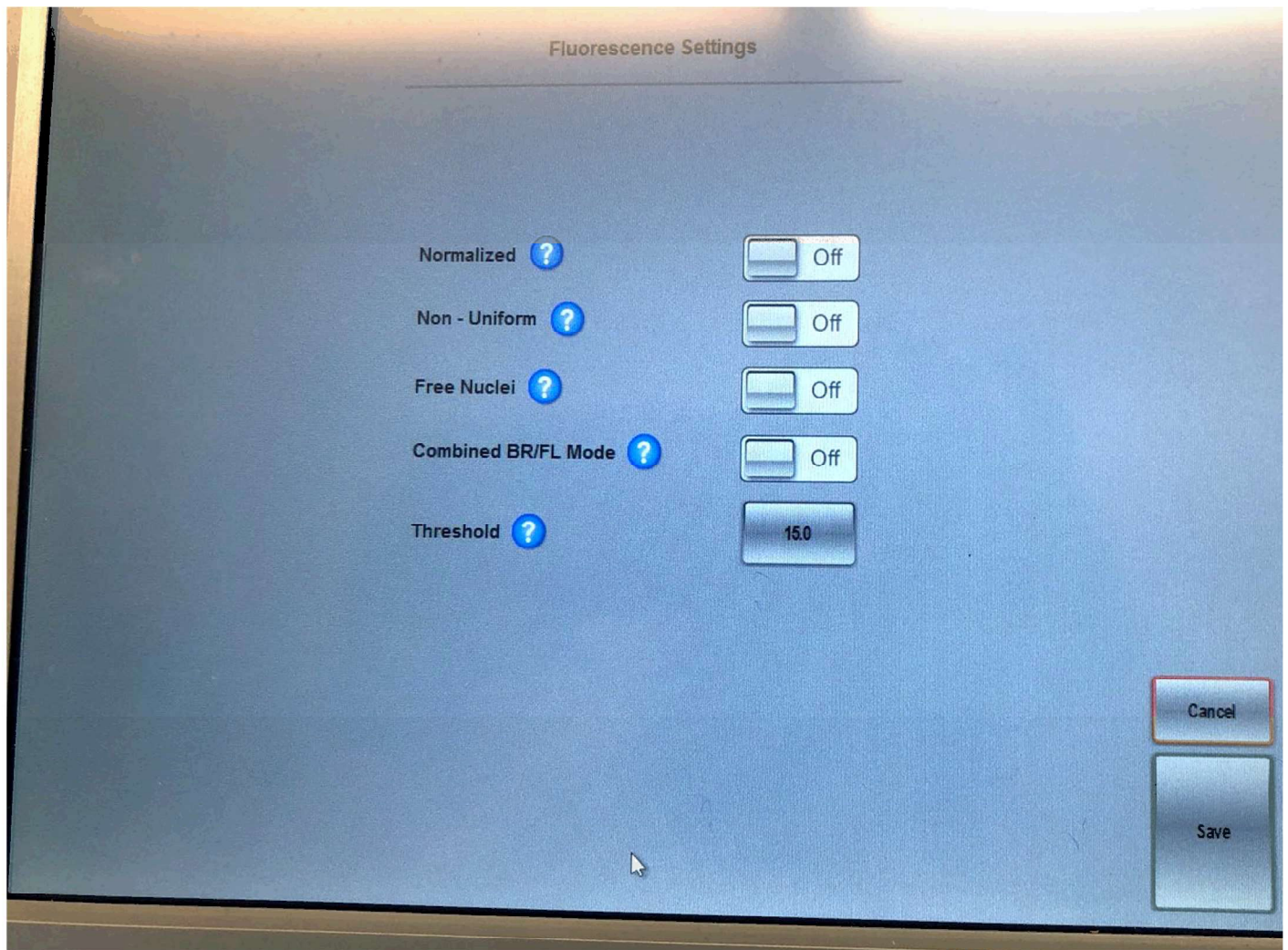
Max Size

☒ On

Advanced Options

Channel 2 Settings – Cell Type Parameters

Click on “FL” to the right of **Advanced Options**.



Channel 2 Settings – Fluorescence Settings

Confirm the settings match those above. **Threshold** should equal 15.0 for Channel 2, and **Combined BR/FL Mode** is OFF.

Click on SAVE if changes were made or CANCEL.

Channel 2 Settings – Cell Type Parameters


Confirm the settings match those pictured above and that the correct Cell Type is listed (for this assay: Thawed MSC Ch2)

Min Cell Diameter should equal 10.0 and **Max Cell Diameter** should equal 25.0

If modifying an unlocked Cell Type, it is recommended to change the cell type name in this window by clicking on **Edit** to the right of Cell Type and enter a new Cell Type Name below Cell Name.

To Lock the Cell type, in the same edit Cell Type window, slide the option to “Lock Cell Type from Future Editing” to ON. Once prompted, click on **LOCK**. Click on **SAVE**. This step will need to be completed for both Channel 1 and Channel 2 settings.

Assay Parameters



THAWED MSC
AO/PI (CS2-0106) or equivalent
Primary cells, cell lines or cell sample
from dissociated tissues with debris.

Unlocked for Editing

Edit

Print

Imaging Mode: Two Fluorescent Stain

Edit

Channel 1

Cell Type: Thawed MSC Ch1
Stain: AO Live Cells
Light Source: 470 nm
Peak Detection: 535 nm

Edit

Channel 2

Cell Type: Thawed MSC Ch2
Stain: PI Dead Cells
Light Source: 540 nm
Peak Detection: 605 nm

Edit

FL Exposure Time

800 msec.

FL Exposure Time

3.0 sec.

Advanced Settings

Edit

Calculation: $F1/(F1 + F2) * 100\%$
Reports

Edit

Save

Click on **Advanced Settings**

Advanced Settings

Channel 1

Use BR Exposure Time Factor ? ☐ Off
Exposure Factor

Channel 2

Use BR Exposure Time Factor ? ☐ Off
Exposure Factor

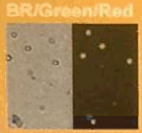
Special Cells ? ☐ Off
Slide Type: CHT4-xD100 ?
Adipocyte ? ☐ Off
Low Concentration ? ☐ Off
Use Default Dilution Factor ? ☒ On
Dilution Value

Advanced Settings

Confirm the settings listed match those pictured above. Click CANCEL or SAVE if changes are made.

Next to **Calculations**, click on “EDIT”

Assay Parameters



BR/Green/Red

THAWED MSC
AO/PI (CS2-0106) or equivalent
 Primary cells, cell lines or cell sample from dissociated tissues with debris.

Unlocked for Editing

Imaging Mode: **Two Fluorescent Stain**

Channel 1

Cell Type: Thawed MSC Ch1
 Stain: AO Live Cells
 Light Source: 470 nm
 Peak Detection: 535 nm

Channel 2

Cell Type: Thawed MSC Ch2
 Stain: PI Dead Cells
 Light Source: 540 nm
 Peak Detection: 605 nm

FL Exposure Time

FL Exposure Time

Advanced Settings

Calculation: $F1/(F1 + F2)*100\%$
 Reports

Calculations and Reports

Formula: $F1 / F1 + F2$ Concentration

Use Custom Formula Label ☒ On

% Viability

Select Formula

- $F1 / F1 + F2$ Concentration
- $F2 / F1 + F2$ Concentration
- $F1 / F2$ Concentration
- $F2 - F1 / F2$ Concentration
- $F2 / F1$ Concentration
- $F1 - F2 / F1$ Concentration

Result Template:
Template_F1-F2-Primary Cells.rlt_tm

Print Template:
Template_F1-F2-Primary Cells.prn_tm

Change Default Edit New

Change Default Edit New

Cancel Save

Calculations and Reports Settings

Confirm the settings match those pictured above.

Click CANCEL or SAVE if changes were made.

Assay Parameters

Assay Icon: **aTHAWED_MSC P2**
 AOPI (CS2-0106) or equivalent
 Primary cells, cell lines or cell sample from dissociated tissues with debris.

Unlocked for Editing

Imaging Mode: Two Fluorescent Stain

Channel 1
 Cell Type: aTHAWED_MSC P2_Ch1
 Stain: AO Live Cells
 Light Source: 470 nm
 Peak Detection: 535 nm

Channel 2
 Cell Type: aTHAWED_MSC P2_Ch2
 Stain: PI Dead Cells
 Light Source: 540 nm
 Peak Detection: 605 nm

FL Exposure Time: 800 msec

FL Exposure Time: 3.0 sec

Advanced Settings
 Calculation: $F1/(F1 + F2) \cdot 100\%$
 Reports

Buttons: Edit, Print, Cancel, Save

If revising an assay, once all changes have been made, next to “Unlocked for Editing”, click on “EDIT”

Assay Descriptions

Assay Icon: **Assay Name**: aTHAWED_MSC P2

Description 1: AOPI (CS2-0106) or equivalent

Description 2
 Line1: Primary cells, cell lines or cell sample from dissociated tissues with debris
 Line2: <blank>
 Line3: <blank>
 Line4: <blank>

Demo Image: Auto2K4-Large Primary Cells-1-BR-A.png

Lock Assay from Future Editing: Off

Buttons: Change, Cancel, Save

Select to **LOCK** the Assay and click “SAVE”

The **printed settings** for **JK THAWED MSC Assay** are as follows:

Assay Type: JK THAWED MSC

Description: Primary cells, cell lines or cell sample from dissociated tissues with debris.
11/16/2020 16:31:06

Imaging Mode: Fluorescent 1 and Fluorescent 2

Single Chamber Assay

Multimode FL Counting not checked

Slide Type: CHT4-xD100

F1 Cell Type: Thawed MSC Ch1

F1

F1 Filterset: AEM-535-001

F1 Lamp: AEX-470-001

F1 Fluorophore: AO Live Cells

F1 Fluorescent Exposure Time: 800.0 msec

F2 Cell Type: Thawed MSC Ch2

F2

F2 Filterset: AEM-605-001

F2 Lamp: AEX-540-001

F2 Fluorophore: PI Dead Cells

F2 Fluorescent Exposure Time: 3000.0 msec

Count Report Format

Formula = $F1 / (F1 + F2) * 100\%$

Use Custom label: Y

Set Dilution Factor for Assay to 2.000

Acquire Brightfield Image checked

Cell Type: Thawed MSC Ch1

Description:

11/16/2020 16:31:06

Brightfield Parameters

Min Cell Diameter: 5.0 microns

Max Cell Diameter: 40.0 microns

Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle

Contrast Enhancement: 0.40 *default 0.40, range: 0 - 0.5; high values for light cells

Decluster Cells

Decluster Edge Factor: 0.5 *default 0.50, range: 0 - 1.0; high values for more edge enhancement

Decluster Th Factor: 1.0 *default 1.0, range: 0 - 1.0; high values for more sensitivity

Background Adjustment: 1.0 *default 1.0, range: 0 - 1.0; lower value to pick up dim cells

Trypan Blue Parameters

Min Cell Diameter: 5.0 microns

Max Cell Diameter: 40.0 microns

Sensitivity: 1.0 *default 1.0, range: 0 - 2.0; higher value to pick up dead cells

Uniformity: 150 *default 150, range: 100 - 255; higher value for non-uniform dead cells

Very Dim Dead Cells not Checked

Fluorescent Parameters

Min Cell Diameter: 5.0 microns

Max Cell Diameter: 40.0 microns

Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle

Decluster Th Factor: 1.00 *default 1.0, range: 0 - 1.0; high values for more sensitivity

Manual Threshold (Based upon sensor intensity range)

Threshold Adjustment: 8.0% *default 80, range: 1 - 100%; lower value to pick up dimmer cells

Sum the cell intensity over the entire cell

Non-uniform cell fluorescence not checked

Use a combined BR and FL image for counting

Cell Type: Thawed MSC Ch2

Description:

11/16/2020 16:31:06

Brightfield Parameters

Min Cell Diameter: 10.0 microns

Max Cell Diameter: 25.0 microns

Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle

Contrast Enhancement: 0.40 *default 0.40, range: 0 - 0.5; high values for light cells

Decluster Cells

Decluster Edge Factor: 0.5 *default 0.50, range: 0 - 1.0; high values for more edge enhancement

Decluster Th Factor: 1.0 *default 1.0, range: 0 - 1.0; high values for more sensitivity

Background Adjustment: 1.0 *default 1.0, range: 0 - 1.0; lower value to pick up dim cells

Trypan Blue Parameters

Min Cell Diameter: 10.0 microns

Max Cell Diameter: 25.0 microns

Sensitivity: 1.0 *default 1.0, range: 0 - 2.0; higher value to pick up dead cells

Uniformity: 150 *default 150, range: 100 - 255; higher value for non-uniform dead cells

Very Dim Dead Cells not Checked

Fluorescent Parameters

Min Cell Diameter: 10.0 microns

Max Cell Diameter: 25.0 microns

Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle

Decluster Th Factor: 1.00 *default 1.0, range: 0 - 1.0; high values for more sensitivity

Manual Threshold (Based upon sensor intensity range)

Threshold Adjustment: 15.0% *default 80, range: 1 - 100%; lower value to pick up dimmer cells

Sum the cell intensity over the entire cell

Non-uniform cell fluorescence not checked

Use the FL image for counting

Signature Manifest**Document Number:** STCL-EQUIP-022 JA2**Revision:** 01**Title:** Cellometer Program Used for Thawed MSC Products JA2**Effective Date:** 04 Mar 2021

All dates and times are in Eastern Time.

STCL-EQUIP-022 JA2 Cellometer Program Used for Thawed MSC Products JA2**Author**

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Document Release

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