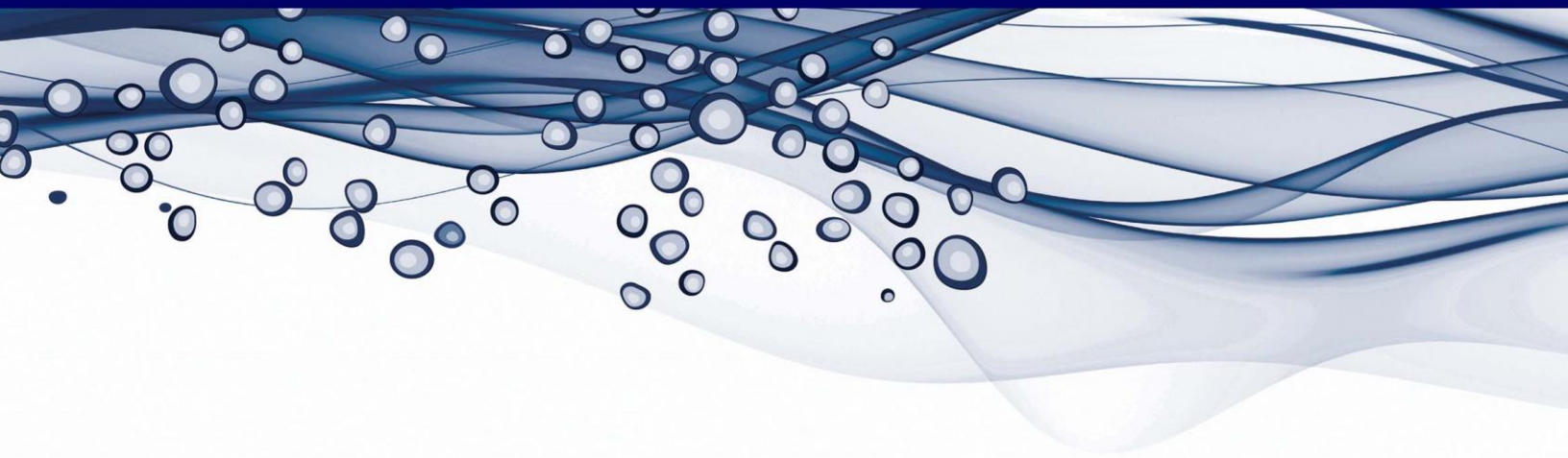
Product Number: CS1-0119

Description: Nexcelom ViaStain™ Calcein AM

Instrument (s): Vision10x, X2, K2, A2K, Vision, Celigo

Instruction Booklet: Nexcelom ViaStain™ Calcein AM



This product is for REASEARCH USE ONLY and is not approved for diagnostic or therapeutic use.

8001614 Rev. C

Product

Part Number: **CS1-0119**

Description: Cellometer ViaStain™ Calcein AM

Size: Cellometer – 100 tests, Celigo – (20) 96-well plates

Description

Calcein AM (Calcein acetoxymethyl ester) is a cell permeable, non-fluorescent compound. Upon crossing the cell membrane, calcein-AM is rapidly hydrolyzed by cellular esterases inside live cells. The hydrolysis cleaves the AM group, converting the non-fluorescent calcein AM to a strongly green fluorescing calcein. Because the created calcein is more hydrophilic it is trapped inside the cells with intact membranes. Cells that do not possess active cytoplasmic esterases are unable to convert calcein AM to calcein, and therefore do not fluoresce green. This allows for a quick and easy detection of metabolically-active cells in a sample.

Since calcein-AM does not require DNA binding, it stains all metabolically-active cells and can be used to measure metabolic activity in non-nucleated cells, such as platelets. Because calcein AM is photostable, has low cytotoxicity and does not affect cellular functions, it is a popular stain for the examination of cell vitality and metabolic activity.

Materials

Materials Supplied

1. Nexcelom ViaStain™ Calcein AM (CS1-0119)*

*Each kit contains sufficient material to perform ~ 100 tests using the Cellometer instruments or (20) 96-well plates using the Celigo systems

Materials Required

1. Distilled H_2O
2. Phosphate Buffer Saline (PBS)
3. Micro centrifuge tube
4. Pipette
5. Cellometer counting chamber (SD100 or PD100) or 96 well plates
6. Cellometer Vision 10X, X2, A2K, K2, Vision or CeligoS, Celigo 5ch

Procedure for Nexcelom Cellometer X2, Vision 10X, K2, Auto 2000, and Vision Instruments

Preparation of calcein AM Reagent

1. Pipette 2 µl Calcein-AM (Nexcelom Bioscience CS1-0119) into 18 µl of dH_2O. This is now **calcein AM Solution A**. Mix well by pipetting up and down at least 15 times.

Staining Procedure for Platelets in Whole Blood

1. Add 5 µl of whole blood to 85 µl of 1x PBS. Mix well by inverting the tube several times, and pipetting up and down.
2. Add 5 µl of **calcein AM Solution A** to 45 µl of the diluted whole blood sample.
    a. The total sample dilution is 1:20
3. Gently pipette the sample up and down ten times, then incubate for 20 min at 37°C in the dark.
4. Pipette sample up and down 3 times to mix and then load 20 µL into a counting chamber (if using SD100 slides, peel plastic film off both sides before loading).
5. Place loaded slide on a Kimwipe® and wait 1 min before inserting sample into instrument to allow platelets to settle in the chamber.
6. Preview bright field and fluorescent images.
7. Count

Staining Procedure for Mononuclear Cells in Whole Blood, Cord Blood, and Bone Marrow

1. Pipette 10 µl of fresh blood sample into 70 µl of 1 x PBS.
2. Pipette 45 µl of the diluted blood sample into a new eppendorf tube.
3. Add 5 µl of **calcein AM Solution A** to 45 µl of the diluted blood sample.
4. Gently pipette the sample up and down ten times, then incubate for 20 min at 37°C in the dark.
5. After the 20 minute incubation, the sample is ready for analysis.
6. Pipette sample up and down 3 times to mix and then load 20 µL into a counting chamber (if using SD100 slides, peel plastic film off both sides before loading).
7. Preview bright field and fluorescent images.
8. Count

Staining Procedure for Cultured and Primary Cells

1. Add 5 µl of **calcein-AM Solution A** to 45 µl of cell sample that is at a concentration of $2x10^6$ cells/ml.
2. Gently pipette the sample up and down ten times, then incubate for 20 min at 37°C in the dark.
3. After the 20 minute incubation, the sample is ready for analysis.
4. Pipette sample up and down 3 times to mix and then load 20 µL into a counting chamber (if using SD100 slides, peel plastic film off both sides before loading).
5. Preview bright field and fluorescent images.
6. Count

Procedure for Nexcelom Celigo Instruments

Preparation of calcein AM Reagent

1. Pipette 10 µl calcein AM (Nexcelom Bioscience CS1-0119) into 20 mL of PBS to stain one 96-well plate at a volume of 200 µl/well.

Staining Procedure for Cultured and Primary Cells

2. Mix and add 200 µl of staining solution per well.
3. Incubate for 20 minutes at 37°C in the dark.
4. Place plate into the Celigo.
5. Set up imaging parameters.
6. Image

Storage and Handling

Store Nexcelom ViaStain™ Calcein AM between -24°C and -16°C . Please consult the Material Safety Data Sheet for more safety information, found on www.nexcelom.com/Products.

Warranty

This product is for RESEARCH USE ONLY and is not approved for diagnostic or therapeutic use. Product is warranted to meet the specifications outlined in the Certificate of Analysis when stored and used according to the manufacturer's instructions. No other warranty, expressed or implied (such as merchantability, fitness for a particular purpose, or non-infringement) is granted. Warranty is valid until the expiration date stated on the product label. If no expiration is listed, the warranty is valid for 12 months from the date of product receipt.

Warranty will be void if product is stored incorrectly, the recommended protocol is not followed, or the product is used for a different application.

Ordering Information

When ordering with a Purchase Order:

Fax a copy of the order to 978-327-5341

Email a copy of the order to sales@nexcelom.com

When ordering with a Credit Card:

Visit www.shop.nexcelom.com and place your order