

User Guide

SMC® Fluorescence Verification Kit

For Instrument Verification of Fluorescence

03-0209-00

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Introduction

The SMC[®] Fluorescence Verification Kit is comprised of two sets of fluorescently labeled molecules and is used in the monthly fluorescence verification of the FemtoQuest[™] Instrument and routine performance verification. Two separate concentrations are included as Verification A (High Standard) and Verification B (Low Standard). The user pipettes 30 μ L of reagent into the SMC[®] 384-well Read Plate as described (see plate layout) and the plate is loaded into the FemtoQuest[™] System. The labeled molecules are detected and counted. The number of fluor-labeled antibodies counted is directly proportional to the nominal concentration of the reagent. Results are analyzed by the instrument software and used to determine the operational status of the FemtoQuest[™] Instrument.

Supplies

The SMC[®] Fluorescence Verification Kit includes all reagents listed below. Additional reagents and supplies are required to run this kit, as listed in the next section, Additional Supplies Required (Not provided).

This kit and all reagents supplied are for research use only.

Reagents Included with the Kit

All items are shipped with a cold pack unless otherwise stated.

Description	Storage Conditions	Packaging Details	Component Number
Verification A	2-8 °C	5 x 1 mL	02-0209-01
Verification B	2-8 °C	5 x 1 mL	02-0209-02
SMC [®] 2 Plex Commercial Read Plate	RT	5 x 1 EA	02-2PCP-00

Storage Instructions

The SMC[®] Fluorescence Verification Kit should be stored at 2-8 °C. Proper kit performance can only be guaranteed if the materials are stored properly.

Discard Verification A and B after each use.

Additional Supplies Required (Not provided)

Catalogue numbers are provided to purchase products at [SigmaAldrich.com](https://www.sigmaaldrich.com) or through sales quote, unless otherwise noted.

Equipment

- FemtoQuest™ Ultrasensitive Immunoassay System for sample acquisition (95-0200-00)
- Fluorescence Verification Kit Pipet Guide (700201-09)
- Rotisserie tube rotator for reagent suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x g for plate centrifugation
- Single channel manual pipettes to accurately dispense 30 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

Operational Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC® assays. The following notes should be reviewed and understood before the kit is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20-25 °C.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- As the SMC® instrument and assay are extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- After the plating is complete, seal the plate before reading immediately or storing temporarily at 2-8 °C. The FemtoQuest™ Immunoassay System requires the use of aluminum adhesive plate seal.
- If SMC® Read Plate has been stored at 2-8 °C, plate should be left at RT, 20-25 °C for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC® Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x g for 1 minute prior to reading.
- SMC® Fluorescence Verification plates should be discarded after use.
- For optimal FemtoQuest™ Immunoassay System performance, perform Self-Test daily and SMC® Fluorescence Verification Kit monthly.

Precautions

Wear protective clothing and gloves.

Components of this reagent kit contain sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

Preparation

Reagent Preparation

1. Warm all reagents to RT prior to use.
2. Place the vial of Verification A (High Standard) and the vial of Verification B (Low Standard) on the rotisserie tube rotator \geq 30 minutes to ensure it is fully homogenized.

Operating Procedure

Plating

1. Remove one vial of each reagent from the original packaging (each vial contains sufficient reagent to prepare one verification plate).
2. Open one tube at a time, place the Fluorescence Verification kit pipet guide onto the SMC[®] 2 Plex Commercial Read Plate and pipette 30 μ L of Verification A per well in High Standard wells and 30 μ L of Verification B per well in Low Standard wells.
 - It is recommended to pipette Verification B first then Verification A to minimize potential cross-contamination.
 - It is recommended to reverse pipette reagents.
3. Once the verification plate is plated, wipe down the pipette guide to remove residual reagent.

To read on the FemtoQuest™ Immunoassay System

1. Firmly seal the SMC[®] Read Plate with a new clear adhesive plate seal using. Then centrifuge the plate for 1 minute at RT, approximately 1,100 x g. Remove the seal, inspect reading plate wells and remove bubbles if they are present.
2. Firmly seal the reading plate with aluminum plate seal using the recommended plate roller.
3. Open the FemtoQuest™ Runner Software and select Run Verification Plate.
4. Select Open Tray.
5. Remove the plate holder from the sealed reading plate and load it onto the FemtoQuest™ Immunoassay System.
6. Select Close Tray then Start Read.

Note: There is a warmup period of up to 30 minutes to equilibrate plate temperature to internal instrument temperature. Once achieved the read will start automatically.

Analyzing Results

1. Select Show More under SMC[®] Fluorescence Verification Kit Results.
2. The Show More screen has options to select between High (Verification A)/Low (Verification B) and Channel 1/Channel 2.

A Levy-Jennings plot of historical runs is displayed on this menu.
3. Select Save Results as PDF.
4. If the verification results display a Fail result, please refer to the trouble-shooting section.

Performance Characteristics

Precision

The precision is evaluated as %CV for each reagent in each channel.

- Mean intra-reagent variation should be < 4% for Channel 1, High.
- Mean intra-reagent variation should be < 8% for Channel 1, Low.
- Mean intra-reagent variation should be < 5% for Channel 2, High.
- Mean intra-reagent variation should be < 9% for Channel 2, Low.

Example Levy-Jennings Plot

The FemtoQuest™ Runner Software displays the results of the High (Verification A) and Low (Verification B) reagents in both Channel 1 and Channel 2 in the form of a Levy-Jennings plot. This plot displays the accumulated results of the high and low reagent in both channels across multiple runs. The blue center line represents the mean Response value. The red lines above and below this represent Mean + 3SD and Mean - 3SD response. It is a succinct reference for historical results of the Fluorescence Verification Kit that aids a user in assessing instrument performance.

In Figure 1 (run #13) and Figure 4 (run #22), show an outlier value that surpasses the Mean + 3SD Response boundary.

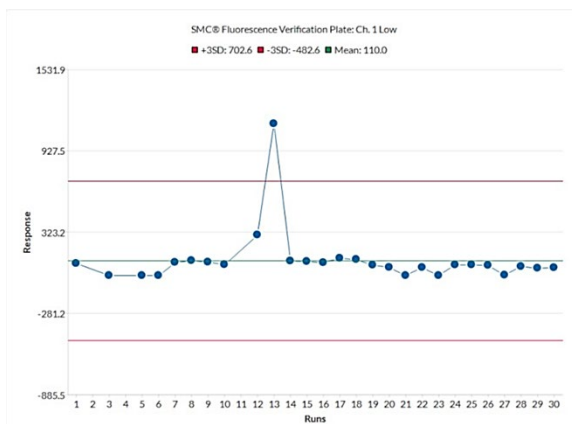


Figure 1: Channel 1 – Low

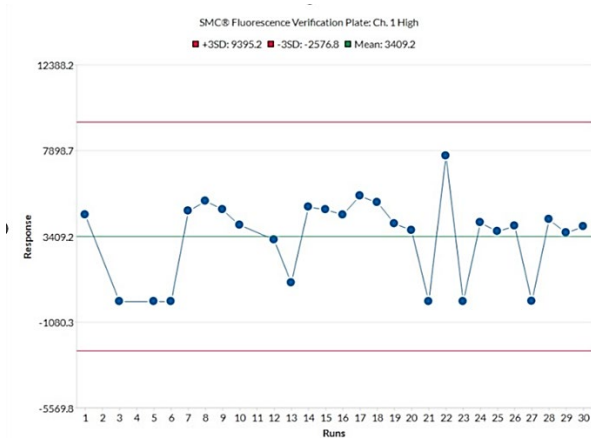


Figure 2: Channel 1 – High

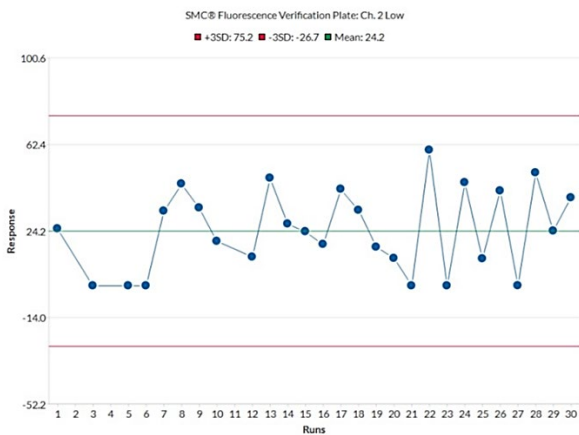


Figure 3: Channel 2 – Low

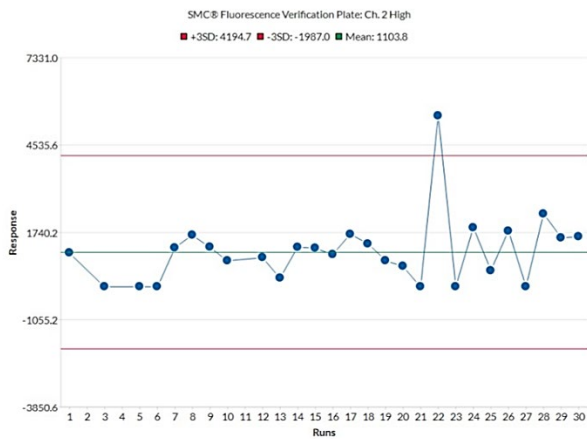


Figure 4: Channel 2 – High

Troubleshooting

Problem	Probable Cause	Solution
Reagent %CV is too high	Vial was not sufficiently homogenized	Adequately rotate reagent vials prior to plate addition
	Verification B wells were contaminated with traces of Verification A	Pipette Verification B prior to Verification A Clean pipet guide before and after use
	Pipette used not properly calibrated or functioning	Use calibrated, functioning pipettes
	Unknown failure	Discard reagent and retrieve new, unopened vials and rerun
Reagent RE value is out of range	Reagent may not have been stored per recommendations	Discard reagent and retrieve properly stored vials
	Verification Reagents not protected from light	
	Verification Reagents plated outside of provided plate-map	Use provided pipet guide
	Pipette used not properly calibrated or functioning	Use calibrated, functioning pipettes

Well Plate

384	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		A	A	A	A	A	A	A	A	A	A	
C		A	A	A	A	A	A	A	A	A	A	
D												
E		B	B	B	B	B	B	B	B	B	B	
F		B	B	B	B	B	B	B	B	B	B	
G												
H												

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Contact Information

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23208541 Rev 04/25

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