

Quick Reference Card

Amnis[®] Protein Aggregate and Silicone Oil Detection Kit

APH10001

Two color assay kit for detection and discrimination of silicone oil droplets and protein aggregates using a convenient mix-and-read assay.

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions

Store kit components at $\leq -20^{\circ}\text{C}$. Store positive and negative IgG controls at $2-8^{\circ}\text{C}$ upon reconstitution with water.

Kit Components

- 1000X ProteoStat[®] Protein Aggregate Detection Reagent (Part Number 4700-1681, 20 μL , 400 tests/vial)
- 1000X PMPBF2 Silicone Oil Detection Reagent: (Part Number 4700-1688, 20 μL , 400 tests/vial)
- Aggregated IgG Positive Control (Part Number 4700-1685, 100 μg , 20 tests/vial)
- Monomeric IgG Negative Control (Part Number 4700-1673, 100 μg , 20 tests/vial)
- 10X Assay Buffer (Part Number 4700-1680, 5 mL/vial)
- Nuclease Free Water (Part Number 4700-1684, 5 mL/vial)

Materials Recommended

- ImageStream[®] or FlowSight[®] imaging cytometer
- SpeedBead[®] ImageStream[®] System Calibration Reagent (Cat # 400041) or FlowSight[®] Calibration Beads (Cat # 400300)
- 10% Bleach
- Samples of interest
- Microcentrifuge tubes
- Micropipettes
- Disposable presterilized filtered micropipette tips
- Silicone Oil (Sigma Aldrich, CAS: 0063148629)
- Centrifugal Filters (EMD Millipore, Cat # UFC30VV25)

Assay Protocol

1. Prepare samples (45 μL each)
 - Experimental samples
 - Silicone oil control
 - Positive and negative IgG control
 - Buffer only control
 - Single-color compensation controls
2. Prepare 10X staining reagents:
 - 10X ProteoStat[®]/PMPBF2 Staining Buffer
 - 10X ProteoStat[®] Single-Color Staining Buffer
 - 10X PMPBF2 Single-Color Staining Buffer

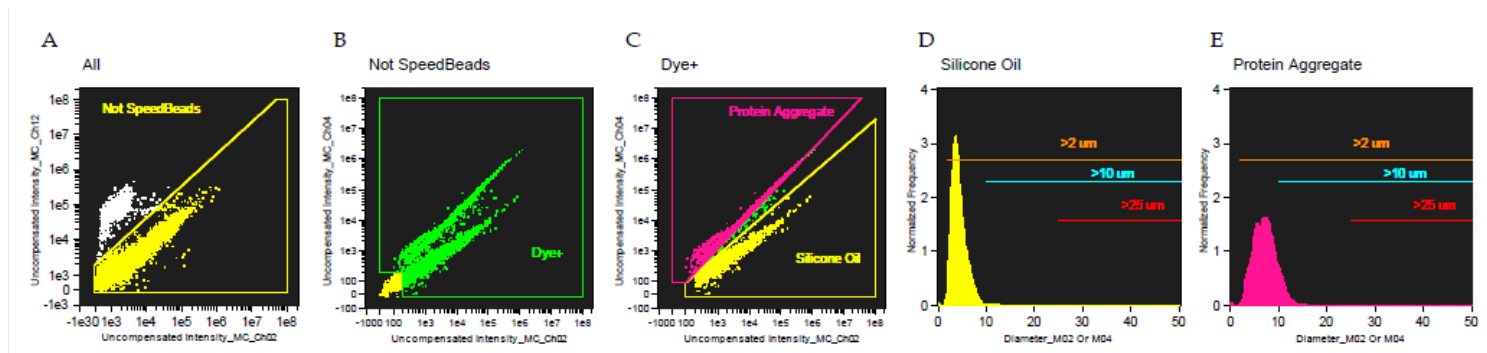
*It may be necessary to further dilute 10X buffers into 1X Assay Buffer based on titration data.
3. Stain samples
 - Add 5 μL of appropriate 10X Single-Color Staining Buffer to single-color compensation controls
 - Add 5 μL 10X ProteoStat[®]/PMPBF2 Staining Buffer to remaining samples
 - Mix each sample gently with a pipette, and incubate 15 minutes at room temperature
4. Run samples on the ImageStream[®] or FlowSight[®]
5. Analyze data files in IDEAS[®] using analysis template provided

Analysis in IDEAS®

Figure 1 shows an example analysis for protein aggregate and silicone oil quantitation using the IDEAS® analysis template provided.

Figure 1: Sequence of Analysis Steps for ImageStream®

A Gate out SpeedBeads. **B** Gate events positive for PMPBF2 or ProteoStat®. **C** Identify Protein Aggregate and Silicone Oil Populations **D** Measure Silicone Oil Size Distribution. **E** Measure Protein Aggregate Size Distribution.

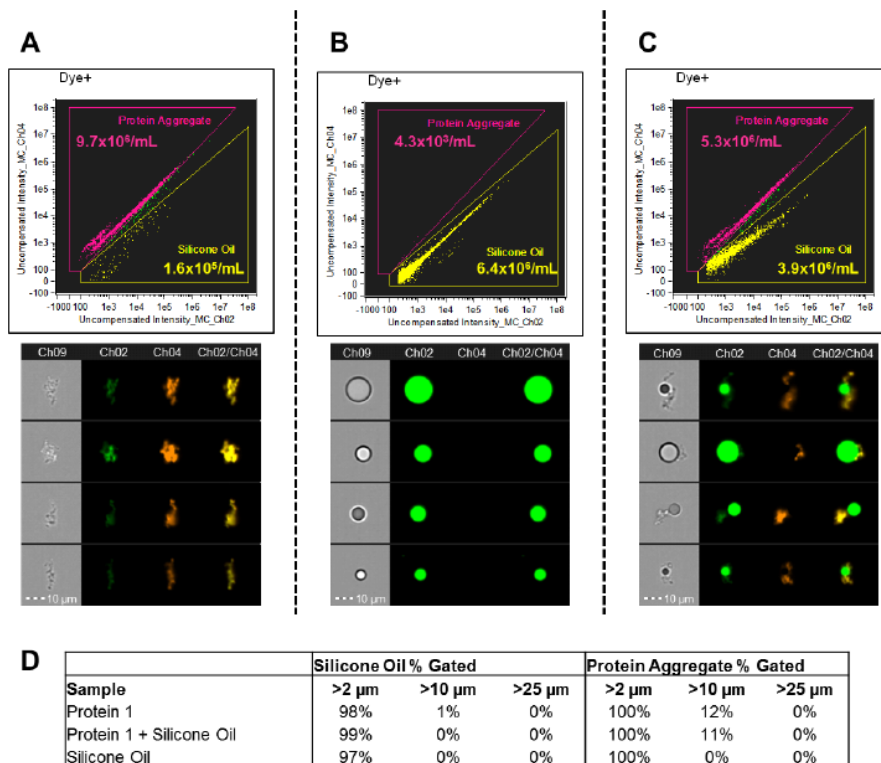


Expected Results

Figure 2 shows an example of results obtained using the Protein Aggregate and Silicone Oil Detection Kit.

Figure 2: Example ImageStream Data

Scatter plot of Uncompensated Intensity_MC_Ch02 vs. Uncompensated Intensity_MC_Ch04 and representative images for (A) Protein 1 (stressed IgG, 30 μg/mL), with images of aggregates, (B) Silicone Oil, with images of silicone oil droplets (0.02%), (C) a 50:50 mixture, with images of heterogeneous complexes containing both aggregate and silicone oil droplet. (D) Size distribution analysis.



A detailed kit user's guide can be found at www.luminexcorp.com/flowkits.

To place an order or receive technical assistance, please visit: support@luminexcorp.com

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