

User Guide

SMC[®] Human NF-L High Sensitivity Immunoassay Kit

Human NF-L Immunoassay Kit for the Quantitative Determination of NF-L in Human Serum, Plasma and CSF

03-0202-00

**FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES**

| | | | |
|--|---|---|----|
| Introduction | 2 | Assay Reading on the SMCxPRO [®] Immunoassay System..... | 10 |
| Supplies..... | 2 | Assay Characteristics..... | 10 |
| Included with the Kit | 2 | Typical Reference Curve..... | 10 |
| Kit Storage..... | 3 | Sensitivity | 10 |
| Additional Supplies Required | 3 | Precision | 11 |
| Assay Best Practices | 4 | Cross-Reactivity | 11 |
| SMC [®] Assay Overview | 5 | Spike Recovery | 11 |
| Assay Preparation | 6 | Troubleshooting..... | 12 |
| Sample Preparation..... | 6 | Precautions | 13 |
| Sample Dilution | 6 | Safety Data Sheets (SDS) | 13 |
| Reagent Preparation..... | 6 | Hazard Labels | 14 |
| Initial Standard Stock Preparation | 6 | Notice | 16 |
| Standard Curve | 7 | Technical Assistance | 16 |
| Assay Procedure | 8 | Terms and Conditions of Sale... .. | 16 |
| Target Capture | 8 | Contact Information | 16 |
| Post-Capture Wash | 8 | | |
| Detection Antibody Incubation ... | 8 | | |
| Post-Detection Wash | 8 | | |
| Post-Detection Shake | 9 | | |
| Final Aspiration..... | 9 | | |
| Elution..... | 9 | | |

Introduction

The SMC[®] Human NF-L High Sensitivity Immunoassay Kit utilizes Single Molecule Counting technology, based on the trusted sandwich immunoassay principle, to digitally measure Neurofilament Light Chain Protein (NF-L) in Human Serum, Plasma and Cerebrospinal Fluid (CSF) samples. A capture antibody specific for Human NF-L has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the NF-L present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to NF-L that has been captured onto the beads, thus completing the immunosandwich. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the beads surface releasing the labeled antibodies. These antibodies are separated during transfer to a 384-well read plate. The plate is loaded into the SMCxPRO[®] System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of NF-L present in the sample when captured. The amount of NF-L in unknown samples is interpolated from a standard curve.

Supplies

Included with the Kit

The SMC[®] Human NF-L High Sensitivity Immunoassay Kit includes all reagents listed below; these components are not intended to be purchased or used individually. Additional reagents and supplies are required to run this immunoassay, as listed in the next section; Additional Supplies Required (not provided). This kit and all reagents supplied are for research use only.

| Description | Shipping Conditions | Storage Conditions | Packaging Details | Component Number |
|---------------------------|---------------------|--------------------|--------------------|------------------|
| 1 Assay Buffer | with cold pack | 2-8 °C | 2 x 20 mL | 02-9955-00 |
| 2 NF-L Coated Beads | with cold pack | 2-8 °C | 1 x 550 µL | 02-2202-00 |
| 3 Standard Diluent | with cold pack | 2-8 °C | 2 x 20 mL | 02-9956-00 |
| 4 NF-L Detection Antibody | with cold pack | 2-8 °C | 1 x 270 µL | 02-1202-00 |
| 5 NF-L Standard | with cold pack | 2-8 °C | 1 lyophilized vial | 02-8202-00 |
| 6 10X Wash Buffer | with cold pack | 2-8 °C | 2 x 50 mL | 02-0001-03 |
| 7 Buffer D | with cold pack | 2-8 °C | 1 x 6 mL | 02-0446-00 |
| 8 Elution Buffer B | with cold pack | 2-8 °C | 1 x 5 mL | 02-0211-02 |

For research use only. Not for use in diagnostic procedures.

Kit Storage

The SMC[®] Human NF-L High Sensitivity Immunoassay Kit should be stored at 2-8 °C. Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup[®] filter, for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X wash buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

Additional Supplies Required (not provided)

Catalogue numbers are provided to purchase products at SigmaAldrich.com unless otherwise noted.

Equipment

- SMCxPRO[®] Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- Orbital microplate shaker for assay plate incubation (e.g. Boekel Scientific[®] Jitterbug™)
- Bio-Tek[®] 405 TSUVS Microplate Washer for assay plate washing (95-0004-05)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x g for sample/plate centrifugation
- Microcentrifuge capable of reaching 13,000 x g for reagent/sample centrifugation
- Single channel P20 and P250 manual pipettes
- 12-channel P20 and P250 manual pipettes
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

Supplies

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for wash buffer dilution
- Stericup[®] Filter, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen[®]_{HTS} BV 96-Well Filter Plate for sample filtration (MSBVN1210)
- 15 mL conical tube with cap for capture bead and detection antibody dilution
- 96-well V-bottom plate for assay setup (Fisher Scientific, 14-222-241)
- Nunc™ Clear Adhesive Plate Seal for assay plate sealing (Fisher Scientific, 236366)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel Reagent Reservoir (sterile) for standard serial dilution (Argos/Cole Parmer 04395-33)

For research use only. Not for use in diagnostic procedures.

- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for detection antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for detection antibody filtration (Fisher Scientific, 14-829-45)
- SMCxPRO® 384-well plate with adhesive seal (02-1008-00)
- SMCxPRO® 384-well plate, bulk case of 32 (ABB2-00160A)
- Nunc™ aluminum adhesive plate seals (Fisher Scientific, 276014)

Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- De-ionized or distilled water for dilution of 10X Wash Buffer

Assay 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. The following notes should be reviewed and understood before the assay 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 (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.
 - The standards prepared by serial dilution must be used within 10 minutes of preparation.
- Note:** It is recommended that the standards are prepared as the last step prior to plate setup.
- All washing must be performed with the wash buffer provided.
 - Boekel Scientific® Jitterbug™ Shaker settings #3-#5 provide maximal orbital mixing without splashing liquid or causing cross-contamination.
- Note:** If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination. Plate must also be protected from light after adding detection.
- After the assay is complete, the plate should be read immediately.
 - The plates may be stored at 2-8 °C for up to 48 hours away from light if same day reading is not possible.
 - After the assay is complete, seal the plate before storing at 2-8 °C.
- Note:** For SMCxPRO® Immunoassay System, use aluminum adhesive plate seal
- Bring to RT then centrifuge the plate at 1,100 x g for 1 minute prior to reading.
- For optimal SMCxPRO® performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).

SMC[®] Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 μL of Standard/1:2 diluted Serum/Plasma or 1:4 diluted CSF samples and 100 μL of Coated Beads to assay plate.
3. Seal assay plate and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.



2 hours at 25 °C

4. After capture incubation, centrifuge assay plate at 1,100 $\times g$ for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20 μL of Detection Antibody per well.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.



1 hour at 25 °C

8. Perform Post-Detection Wash.
9. Seal assay plate and perform Post-Detection Shake for 2 minutes on microplate incubator/shaker.
10. Perform Final Aspiration.
11. Remove from washer magnet and add 10 μL of Elution Buffer B to each well.
12. Seal assay plate and incubate for 10 minutes at 25 °C on microplate.



10 minutes at 25 °C

13. Add 10 μL of Buffer D to neutralize the eluted antibody.
14. Transfer 18 μL of neutralized eluate to the **reading plate**.
15. Seal **reading plate** aluminum adhesive plate seal for SMCxPRO[®] System.

Load on SMCxPRO[®] System

Assay Preparation

Sample Preparation

1. Using a filter plate with prefilter, stack the filter plate on top of a 96-well receptacle plate.
2. Place 250 μ L of sample into a filter plate well.
3. Spin for ≥ 10 minutes at 1,100 x g.

Sample Dilution

1. Dilute clarified Serum and Plasma samples 1:2 using the provided Standard Diluent (e.g. for triplicates, transfer 200 μ L of clarified sample to the sample preparation plate and add 200 μ L Standard Diluent).
2. Dilute clarified CSF samples 1:4 using the provided Standard Diluent (e.g. for triplicates, transfer 100 μ L of clarified sample to the sample preparation plate and add 300 μ L Standard Diluent).
3. 100 μ L per well of 1:2 diluted Serum and Plasma or 1:4 diluted CSF should be used.
4. If further sample dilution is required, samples can be diluted with the provided Standard Diluent.

Reagent Preparation

1. Warm all reagents to room temperature (RT) prior to use.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
 - o Pour both bottles of 10X Wash Buffer (containing 50 mL each for 100 mL total) into a container capable of holding at least 1 L. Add 900 mL of deionized water.
 - o Mix thoroughly by gentle inversion or with a clean, sterile stir bar.**NOTE:** 1X Wash Buffer may be filter sterilized (refer to Storage Instructions)
4. Mix NF-L Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

Initial Standard Stock Preparation

Reconstitute lyophilized standard in 250 μ L of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.

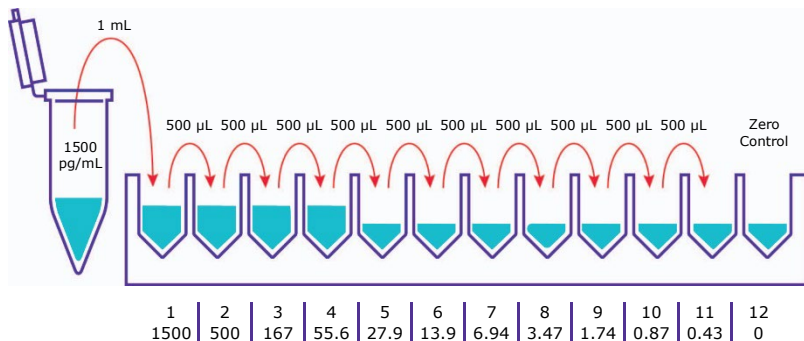
Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the NF-L Standard in the vial.

Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 1,500 pg/mL in a 1.0 mL final volume.

Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 1,500 pg/mL Standard 1 for Standards 2 through 4, and 1:2 serial dilutions of Standards 4 through 11 to achieve a curve from 1,500 pg/mL to 0.43 pg/mL. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.



NOTE: The Standard Diluent used in this assay contains a surfactant. Pipette gently into reservoir wells to avoid creating bubbles.

1. Add 1,000 µL of Standard Diluent to wells 2 through 4 of a 12-channel reservoir dilution plate.
2. Add 500 µL of Standard Diluent to wells 5 through 12.
3. Transfer 1,000 µL of the 1,500 pg/mL working stock (Standard 1) into well 1.
4. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

Assay Procedure

Target Capture

1. Pipette 100 μL per well of Standards, 1:2 diluted Serum or Plasma, or 1:4 diluted CSF Samples to the assay plate.
2. Retrieve the NF-L Coated Bead vial from the rotator and transfer its full contents to 11.0 mL of supplied Assay Buffer. Rinse the bead vial with 0.55 mL of fresh Assay Buffer and ensure that all beads have been transferred from the original vial. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted NF-L Coated Beads.
3. Pipette 100 μL per well of diluted NF-L Coated Beads into the assay plate.
4. Seal the assay plate with clear adhesive plate seal, applying pressure to the seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25 $^{\circ}\text{C}$ on microplate incubator/shaker set at approximately 600-700 RPM or Jitterbug setting #3.
6. **Approximately 10 minutes prior to the end of target capture incubation, prepare the NF-L detection antibody working stock:**
Prepare 1X detection antibody by adding 250 μL of 20X detection antibody into 4,750 μL of Assay Buffer and filter the diluted detection antibody using the syringe with a 0.2 μm filter into a clean tube.
7. When incubation is complete, centrifuge at 1,100 $\times g$ for 1 minute and carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash plate once with a plate washer using the Post Capture Wash (POSTCAP) program on the Bio-Tek[®] 405 TSUVS washer

If using automation, please contact your technical service representative for the appropriate automation procedure.

Detection Antibody Incubation

1. After removal from plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners.
2. Dispense 20 μL per well of 1x NF-L Detection Antibody using reverse pipetting without disturbing the bead pellets.
3. Seal the assay plate with a new clear adhesive plate seal.
4. Incubate for 1 hour at 25 $^{\circ}\text{C}$ on microplate incubator/shaker set at approximately 1000 RPM or Jitterbug setting #5. **Ensure plate is protected from light during this incubation.**
5. When incubation is complete, centrifuge at 1,100 $\times g$ for 1 minute and carefully remove the clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash the assay plate once with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the Bio-Tek[®] 405 TSUVS washer.

If using automation, please contact your technical service representative for the appropriate automation procedure.

Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 μ L of wash buffer.
2. Seal the assay plate with a new clear adhesive plate seal and apply pressure to the seal to prevent leaking and cross-contamination.
3. Place the plate on the microplate/incubator shaker set at approximately 600-700 RPM or Jitterbug setting #3 for 2 minutes. **Ensure plate is protected from light during this incubation.**
4. Remove the plate from the shaker, and centrifuge at 1,100 x g for 1 minute. Carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

Final Aspiration

Perform the Final Aspirate program (FINASP) program on the Bio-Tek® 405 TSUVS washer.

If using automation, please contact your technical service representative for the appropriate automation procedure.

Elution

1. Remove the assay plate and sphere magnet from the BioTek plate washer, and place them on the bench.
2. Place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners.
3. Carefully remove the clear adhesive film and dispense 10 μ L Elution Buffer B per well using reverse pipetting without disturbing the bead pellet.
4. Seal assay plate with a new clear adhesive plate seal.
5. Incubate the plate for 10 minutes at 25 °C on microplate incubator/shaker set at 1000 RPM or Jitterbug setting #5. **Ensure plate is protected from light during this incubation.**
6. When incubation is complete, centrifuge at 1,100 x g for 1 minute and carefully remove the clear adhesive plate seal to avoid splashing.

Assay Reading on the SMCxPRO® Immunoassay System

1. Place the assay plate with Elution Buffer B onto the sphere mag plate and allow beads to form a tight pellet for 2 minutes.
2. Keeping the assay plate on the magnet, add 10 μL of Buffer D to center of wells containing Elution Buffer B. Use a fresh tip with each dispense.
3. Keep the assay plate on the magnet, and set a 12-channel P20 manual pipette to 18 μL and put 12 tips onto the pipettor. Transfer 18 μL of neutralized eluate solution per well to corresponding wells of the SMCxPRO® reading plate with the plate holder underneath. **Avoid touching the beads with the pipet tips, and change tips with each dispensed row.**
4. Seal the reading plate with new clear adhesive plate seal. Centrifuge 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.
5. Firmly seal the reading plate with aluminum plate seal using the recommend plate roller.
6. Remove the plate holder from the sealed reading plate and load it onto the SMCxPRO® Immunoassay System. Start read.

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

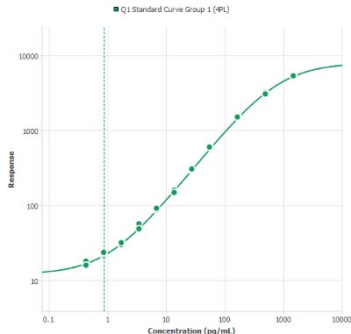
Assay Characteristics

Typical Reference Curve

Typical SMCxPRO® Immunoassay System Standard Curve is shown on right (not to be used to calculate data)

Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of NF-L is 0.87 pg/mL.



Precision

The assay variations of SMC® Human NF-L Immunoassay kits were studied using five normal plasma samples run in triplicate by 3 different operators on 3 different days.

Mean intra-assay variation was 10.2%.

Mean inter-assay variation was 9.6%.

Cross-Reactivity

Cross-reactivity to the following analytes were tested with the following results:

NF-M: Not detected

Total Tau: Not detected

NF-H: Not detected

GFAP: Not detected

pTau (pT181): Not detected

Spike Recovery

The data represent mean percent recovery of three different concentrations of standard spiked into samples (n = 5 Serum samples, 5 Plasma samples, and 5 CSF samples)

| Sample ID | Serum Recovery | Plasma Recovery | CSF Recovery |
|------------------|-----------------------|------------------------|---------------------|
| Sample 1 | 96% | 79% | 130% |
| Sample 2 | 98% | 96% | 115% |
| Sample 3 | 82% | 94% | 126% |
| Sample 4 | 96% | 101% | 130% |
| Sample 5 | 91% | 98% | 118% |
| Average | 93% | 94% | 123% |

Troubleshooting

| Problem | Probable Cause | Solution |
|----------------------------|--|---|
| Background is too high | Background wells were contaminated | Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected |
| | | Ensure reagents (including wash buffer) are not contaminated |
| | | Insufficient washes—washer may need to be cleaned or reprogrammed |
| | Plate was over-incubated | Confirm plate incubation times are as recommended, particularly for the Detection incubation |
| Sample variability is high | Multichannel pipet may not be calibrated | Calibrate pipets |
| | Plate washing was not uniform | Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 μ L or residual remaining in the well |
| | Samples may have high particulate matter or other interfering substances | Samples should be centrifuged or filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision |
| | Plate agitation was insufficient | Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (~650 - 1500 RPM) |
| | Cross-well contamination | Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x g for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed |
| | | Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate |

For research use only. Not for use in diagnostic procedures.

| Problem | Probable Cause | Solution |
|--|--|--|
| Beads are lost during the wash | Plate washer needs optimization/cleaning | Contact Tech Support or local BCS to schedule washer programming. Refer to user guide for cleaning procedure |
| | Insufficiently primed washer | Washer should be primed with wash buffer prior to running the post capture wash protocol |
| | Beads came in contact with water | Washer should be primed with wash buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles |
| | Proper magnet was not used | Ensure that the mag plate catalog number 90-0003-02 was present on plate wash stage prior to running wash protocol |
| Published LLoQ was not achieved | Improper dilution/reconstitution of the standard reference material | Confirm appropriate kit protocol was followed when preparing standard curve |
| | | Check plate washer to confirm no beads were lost during washes and that plate contains < 2 μ L following the post-capture and final aspiration protocols |
| Microparticles do not resuspend into homogenous solution | Beads were not properly stored and may have been frozen | Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly |
| | Samples may be causing interference due to excess particulate matter | Samples should be properly processed prior to testing to remove particulate matter or lipids |

Precautions




Use caution when handling biological samples. Wear protective clothing and gloves.

Components of this reagent kit contain approximately 0.08% 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.

Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at [SigmaAldrich.com](https://www.sigmaaldrich.com).

Hazard Labels

| Ingredient | Component Number | Full Label | |
|-------------------|------------------|--|--|
| NF-L Standard | 02-8202-00 |   | <p>Danger. Harmful if swallowed or if inhaled. Toxic in contact with skin. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Harmful to aquatic life with long lasting effects. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.</p> |
| NF-L Coated Beads | 02-2202-00 | No Label Required | Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. |
| Standard Diluent | 02-9956-00 | No Label Required | Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. |
| 10X Wash Buffer | 02-0001-03 |  | <p>Warning. Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</p> |
| Assay Buffer | 02-9955-00 | No Label Required | Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant. |

| | | | | | | | | | | | | |
|---|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| B | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| C | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 | Standard 8 | Standard 9 | Standard 10 | Standard 11 | Standard 12 |
| D | Sample 1 | Sample 1 | Sample 1 | Sample 2 | Sample 2 | Sample 2 | Sample 3 | Sample 3 | Sample 3 | Etc. | Etc. | Etc. |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

For research use only. Not for use in diagnostic procedures.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck operates
as MilliporeSigma in the U.S. and Canada.

Merck, Millipore, Milliplex, SMCxPRO, Stericup, Multiscreen, Millex and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

For research use only. Not for use in diagnostic procedures.

