# ab211062 Human Neuro Antibody Array I (Membrane, 20 Targets)

Instructions for use:

For quantitative measurement of Human cytokines in a variety of biological samples.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 18 August 2016

# Table of Contents

## 1. BACKGROUND

Abcam's Human Neuro Antibody Array I (ab211062) for use with cell culture media, serum, plasma, cell and tissue lysates and other liquid samples.

Targets: BDNF, beta-NGF, GCSF, GDNF, HB-EGF, IFN gamma, IGF-1, IL-10, IL-1 alpha, IL-1 beta, IL-6, IL-8, MCP-1, MIP-1 alpha, MMP-2, MMP-3, S100B, TGF beta, TNF alpha, VEGF-A.

Cytokine arrays are an antibody-pair-based assay, analogous to ELISA, but using a membrane as a substrate rather than a plate. Capture antibodies are supplied arrayed/spotted on a membrane with each pair of spots representing a different analyte. Sample is added (0.2-1ml of 1 sample to each membrane), and then paired biotinylated detector antibodies and streptavidin HRP. The cytokine array is analyzed using the same methods as a chemiluminescent western blot. Comparison between samples can be by eye or using densitometry software for a semi-quantitative comparison.

# INTRODUCTION

Abcam's Human Neuro Antibody Array I has several advantages over detection of cytokines using single-target ELISA kits:

1. More Data, Same or Less Sample: Antibody arrays provide high-content screening using about the same sample volume as traditional ELISA.

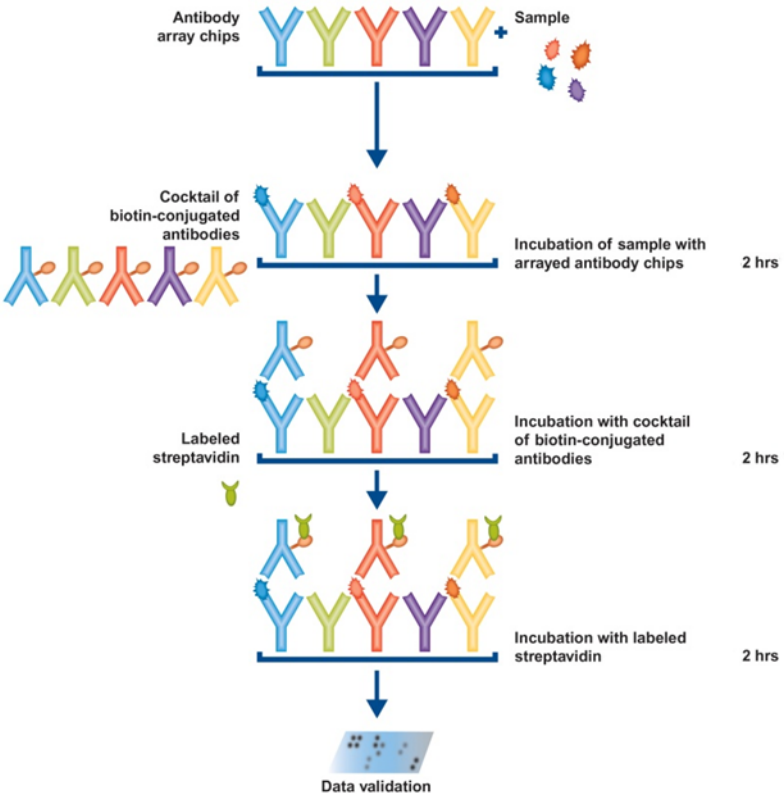
2. Global View of Cytokine Expression: Antibody array screening improves the chances for discovering key factors, disease mechanisms, or biomarkers related to cytokine signaling.

3. Similar (sometimes better) Sensitivity: As little as 4 pg/mL of MCP-1 can be detected using the Membrane array format. In contrast, a similar MCP-1 ELISA assay has a sensitivity of 40 pg/mL of MCP-1.

4. Increased Range of Detection: ELISA assays typically detect a concentration range of 100- to 1000-fold, however, Abcam arrays can detect IL-2 at concentrations of 25 to 250,000 pg/mL, a range of 10,000-fold.

5. Better Precision: As determined by densitometry, the inter-array Coefficient of Variation (CV) of spot signal intensities is 5-10%, comparing favorably with ELISA testing (CV = 10-15%).

## 2. ASSAY SUMMARY



## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. STORAGE AND STABILITY

**Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. MATERIALS SUPPLIED

| Item | Amount (Per Number of Samples) | | | Storage Condition (Before Preparation) | Storage Condition (After Preparation) |
|---|---|---|---|---|---|
| | 2 | 4 | 8 | | |
| Amount (membranes) | 2X | 4X | 8X | -20°C | -20°C |
| Blocking Buffer | 25 mL | 25 mL | 2X 25 mL | -20°C | -20°C |
| Biotinylated Antibody Cocktail | 1 Vial | 2 Vials | 4 Vials | -20°C | 4°C |
| 1,000X HRP-Streptavidin Buffer | 50 µL | 50 µL | 50 µL | -20°C | 4°C |
| 20X Wash Buffer I | 10 mL | 10 mL | 20 mL | -20°C | 4°C |
| 20X Wash Buffer II | 10 mL | 10 mL | 20 mL | -20°C | 4°C |
| 2X Cell Lysis Buffer | 10 mL | 10 mL | 16 mL | -20°C | 4°C |
| Detection Buffer C | 1.5 mL | 1.5 mL | 2.5 mL | -20°C | 4°C |
| Detection Buffer D | 1.5 mL | 1.5 mL | 2.5 mL | -20°C | 4°C |
| 8 Well Incubation tray (with lid) | 1 Unit | 1 Unit | 1 Unit | -20°C | RT |

*Plastic sheets included.

## 7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Pipettors, pipet tips and other common lab consumables.
- Distilled or De-ionized Water.
- Tissue paper, blotting paper or chromatography paper.
- Orbital shaker or oscillating rocker.
- Adhesive tape or plastic wrap.
- A chemiluminescent blot documentation system.

  CCD Camera

  X-Ray Film and a suitable film processor

  Gel documentation system

  Or another chemiluminescent detection system capable of imaging a western blot

## 8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single membrane. The number of samples that can be tested will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

### Handling Array Membranes

- The antibody printed side of each membrane is marked by a dash (-) or number (#) in the upper left corner.
- Do not allow membranes to dry out during the experiment or they may become fragile and break OR high and/or uneven background may occur.
- Grasp membranes by the corners or edges only using forceps. DO NOT touch printed antibody spots

### Incubation and Washes

- Perform ALL incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle per second) using an orbital shaker or oscillating rocker to ensure complete and even reagent/sample coverage. Rocking/rotating too vigorously may cause foaming or bubbles to appear on the membrane surface which should be avoided.

- All washes and incubations should be performed in the Incubation Tray provided in the kit.

- Cover the Incubation Tray with the lid provided during all incubation steps to avoid evaporation and outside debris contamination.

- Ensure the membranes are completely covered with sufficient sample or reagent volume during each incubation.

- Avoid forceful pipetting directly onto the membrane; instead, gently pipette samples and reagents into a corner of each well.

- Aspirate samples and reagents completely after each step by suctioning off excess liquid with a pipette. Tilting the tray so the liquid moves to a corner and then pipetting is an effective method.

- Optional overnight incubations may be performed for the following steps to increase overall spot signal intensities:
  Sample Incubation
  Biotinylated Antibody Cocktail Incubation
  HRP-Streptavidin Incubation

NOTE: Overnight incubations should be performed at 2-8°C (also with gentle rocking/shaking). Be aware that longer incubations can also increase the background response so complete liquid removal and washing is critical.

**Chemiluminescence Detection**

- Beginning with adding the detection buffers and ending with exposing the membranes should take no more than 10-15 minutes as the chemiluminescent signals may start to fade at this point.
- Trying multiple exposure times is recommended to obtain optimum results.
- A few seconds to a few minutes is the recommended exposure time range, with 30 seconds to 1 minute being suitable for most samples.

## 9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening
- Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature and mix gently until dissolved.
- The Biotinylated Antibody Cocktail and the HRP-Streptavidin Concentrate vials should be briefly centrifuged (~1000 x g) before opening to ensure maximum recovery and mixed well as precipitates may form during storage.

9.1. **Biotinylated Antibody Cocktail**

Pipette 2 mL of Blocking Buffer into each vial. Mix gently with a pipette.

9.2. **1,000X HRP-Streptavidin Concentrate**

Dilute 1,000-fold with Blocking Buffer. Mix gently with a pipette.

9.3. **20X Wash Buffer I**

Dilute 20-fold with distilled or deionized water.

9.4. **20X Wash Buffer II**

Dilute 20-fold with distilled or deionized water.

9.5. **2X Cell Lysis Buffer Concentrate**

Dilute 2-fold with distilled or deionized water

9.6. **Blocking buffer**

25 mL and 2X 25 mL. Provided at working strength.

9.7. **Detection Buffer C**

1.5 mL and 2.5 mL. Provided at working strength.

9.8. **Detection buffer D**

1.5 mL and 2.5 mL. Provided at working strength.

9.9. **Antibody arrays**

1 vial of Biotinyated Antibody Cocktail is enough to test 2 membranes.

# 10. SAMPLE PREPARATION

## 10.1. General Considerations

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- Serum-free or low serum containing media (0.2% FBS/FCS) is recommended. If serum containing media is required, testing an uncultured media sample as a negative control is ideal as many types of sera contain cytokines, growth factors and other proteins.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid using EDTA as an anti-coagulant for collecting plasma if testing MMPs or other metal-binding proteins.
- Avoid using hemolyzed serum or plasma as this may interfere with protein detection and/or cause a higher than normal background response.
- Avoid sonication of 1 mL or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.
- Always centrifuge the samples hard after thawing (~10,000 RPM for 2-5 minutes) in order to remove any particulates that could interfere with detection.

## 11. ARRAY MAP

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | POS | POS | NEG | NEG | BDNF | beta-NGF | GCSF | GDNF |
| 2 | | | | | | | | |
| 3 | HB-EGF | IFN gamma | IGF-1 | IL-10 | IL-1 alpha | IL-1 beta | IL-6 | IL-8 |
| 4 | | | | | | | | |
| 5 | MCP-1 | MIP-1 alpha | MMP-2 | MMP-3 | S100B | TGF beta | TNF alpha | VEGF-A |
| 6 | | | | | | | | |
| 7 | BLANK | BLANK | BLANK | BLANK | BLANK | BLANK | BLANK | POS |
| 8* | | | | | | | | |

*Each antibody is spotted in duplicate vertically*

## 12. ASSAY PROCEDURE

**Please prepare all reagents immediately prior to use. All incubations and washes must be performed under gentle rotation/rocking (~0.5-1 cycle/sec). Make sure bubbles do not appear on or between the membranes to ensure even incubations.**

12.1. Remove the kit from storage and allow the components to equilibrate to room temperature (RT).

12.2. Carefully remove the Antibody Arrays from the plastic packaging and place each membrane (printed side up) into a well of the Incubation Tray. One membrane per well.

*NOTE: The antibody printed side is marked by a dash (-) or number (#) in the upper left corner.*

12.3. Pipette 2 mL of Blocking Buffer into each well and incubate for 30 minutes at RT.

12.4. Aspirate blocking buffer from each well with a pipette.

12.5. Pipette 1 mL of diluted or undiluted sample into each well and incubate for 1.5 to 5 hours at RT OR overnight at 4°C.

*NOTE: Longer incubations can help maximize the spot signal intensities. However, doing so can also increase the background response so complete liquid removal and washing is critical.*

12.6. Aspirate samples from each well with a pipette.

*NOTE: The 20X Wash Buffer Concentrates I and II must be diluted 20-fold before use. See Reagent Preparation Section for details.*

12.7. Wash Buffer I Wash: Pipette 2 mL of 1X Wash Buffer I into each well and incubate for 5 minutes at RT. Repeat this 2 more times for a total of 3 washes using fresh buffer and aspirating out the buffer completely each time.

12.8. Wash Buffer II Wash: Pipette 2 mL of 1X Wash Buffer II into each well and incubate for 5 minutes at RT. Repeat this 1 more time for a total of 2 washes using fresh buffer and aspirating out the buffer completely each time.

*NOTE: The Biotinylated Antibody Cocktail must be prepared before use. See Reagent Preparation Section for details.*

12.9. Pipette 1 mL of the prepared Biotinylated Antibody Cocktail into the appropriate well and incubate for 1.5 to 2 hours at RT OR overnight at 4°C.

12.10. Aspirate biotinylated antibody cocktail from each well.

12.11. Wash membranes as directed in Steps 12.7 and 12.8.

*NOTE: The 1X HRP-Streptavidin must be prepared before use. See Reagent Preparation Section for details.*

12.12. Pipette 2 mL of 1X HRP-Streptavidin into each well and incubate for 2 hours at RT OR overnight at 4°C.

12.13. Aspirate HRP-Streptavidin from each well.

12.14. Wash membranes as directed in Steps 12.7 and 12.8.

*NOTE: Do not allow membranes to dry out during detection.*

12.15. Transfer the membranes, printed side up, onto a sheet of chromatography paper, tissue paper, or blotting paper lying on a flat surface (such as a benchtop).

12.16. Remove any excess wash buffer by blotting the membrane edges with another piece of paper.

12.17. Transfer and place the membranes, printed side up, onto a plastic sheet (provided) lying on a flat surface.

*NOTE: Multiple membranes can be placed next to each other and fit onto a single plastic sheet. Use additional plastics sheets if necessary.*

12.18. Into a single clean tube, pipette equal volumes (1:1) of Detection Buffer C and Detection Buffer D. Mix well with a pipette.

*EXAMPLE: 250 µL of Detection Buffer C + 250 µL of Detection Buffer D = 500 µL (enough for 1 membrane)*

12.19. Gently pipette 500 µL of the Detection Buffer mixture onto each membrane and incubate for 2 minutes at RT (DO NOT ROCK OR SHAKE). Immediately afterwards, proceed to Step 12.20.

*NOTE: Exposure should ideally start within 5 minutes after finishing incubation with detection buffer and completed within 10-15 minutes as chemiluminescence signals will fade over time. If necessary, the signals can usually be restored by repeating washing, HRP-Streptavidin and Detection Buffers incubation.*

12.20. Place another plastic sheet on top of the membranes by starting at one end and gently "rolling" the flexible plastic sheet across the surface to the opposite end to smooth out any air bubbles. The membranes should now be "sandwiched" between two plastic sheets.

*NOTE: Avoid "sliding" the top plastic sheet along the membranes' printed surface. If using X-ray film, do not use a top plastic sheet so that the membranes can be directly exposed to the film.*

12.21. Transfer the sandwiched membranes to the chemiluminescence imaging system such as a CCD camera (recommended) and expose.

*NOTE: Optimal exposure times will vary so performing multiple exposure times is strongly recommended.*

12.22. To store, without direct pressure, gently sandwich the membranes between 2 plastic sheets (if not already), tape the sheets together or use plastic wrap to secure them, and store at ≤ -20°C for future reference.

## 13. CALCULATIONS

**Interpreting the Results**

Positive Control Spots (POS) – controlled amount of biotinylated antibody printed onto the array. Used for normalization and to orientate the arrays.

Negative Control Spots (NEG) – buffer printed (no antibodies) used to measure the baseline responses. Used for determining the level of non-specific binding of the samples.

Blank Spots (BLANK) – nothing is printed here. Used to measure the background response.

**Data Extraction**

Visual comparison of array images may be sufficient to see differences in relative protein expression. However, most researchers will want to perform numerical comparisons of the signal intensities (or more precisely, signal densities), using 2-D densitometry. Gel/Blot documentation systems and other chemiluminescent or phosphorescent detection systems are usually sold as a package with compatible densitometry software.

Any densitometry software should be sufficient to obtain spot signal densities from your scanned images. One such software program, ImageJ, is available for free from the NIH website along with an array plug-in.

We suggest using the following guidelines when extracting densitometry data from our array images:

- For each array membrane, identify a single exposure that the exhibits a high signal to noise ratio (strong spot signals and low background response). Strong Positive Control Spot signals but not too strong that that they are “bleeding” into one another is ideal. The exposure time does not need to be identical for each array, but Positive Control signals on each array image should have similar intensities.

- Measure the density of each spot using a circle that is roughly the size of one of the largest spots. Be sure to use the same extraction circle dimensions (area, size, and shape) for measuring the signal densities on every array for which you wish to compare the results.
- For each spot, use the summed signal density across the entire circle (i.e., total signal density per unit area)

**Data Analysis**

Once the raw numerical densitometry data is extracted, the background must be subtracted and the data normalized to the Positive Control signals to analyze.

Background Subtraction: Select values which you believe best represent the background. If the background is fairly even throughout the membrane, the Negative Control Spots (NEG) and/or Blank Spots (BLANK) should be similar and are accurate for this purpose.

Positive Control Normalization: The amount of biotinylated antibody printed for each Positive Control Spot is consistent from array to array. As such, the intensity of these Positive Control signals can be used to normalize signal responses for comparison of results across multiple arrays, much like housekeeping genes and proteins are used to normalize results of PCR gels and Western Blots, respectively.

To normalize array data, one array is defined as "Reference Array" to which the other arrays are normalized to. The choice of the Reference Array is arbitrary.

Next, the simple algorithm below can be used to calculate and determine the signal fold expression between like analytes.

**X(Ny) = X(y) * P1/P(y)**

Where:

P1 = mean signal density of Positive Control spots on reference array

P(y) = mean signal density of Positive Control spots on Array "y"

X(y) = mean signal density for spot "X" on Array for sample "y"

X(Ny)= normalized signal intensity for spot "X" on Array "y"

## 14. TYPICAL DATA

**Typical results obtained with Abcam Antibody Arrays:**


The preceding figure presents typical images obtained with Abcam's Cytokine Antibody Membrane Array. These membranes were probed with conditioned media from two different cell lines. Membranes were exposed to film at room temperature for 1 minute.

Note the strong signals of the Positive Control spots, provided by biotin-conjugated IgG printed directly onto the array membrane in the upper-left and lower-right corners. These Positive Control spots are useful for proper orientation of the array image.

The signal intensity for each antigen-specific antibody spot is proportional to the relative concentration of the antigen in that sample. Comparison of signal intensities for individual antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte sample-to-sample or group-to-group.

## 15. QUICK ASSAY PROCEDURE

***NOTE:*** This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Remove the kit from storage and allow the components to equilibrate to room temperature (RT).
- Pipette 2 mL of Blocking Buffer into each well and incubate for 30 minutes at RT. Aspirate blocking buffer from each well.
- Pipette 1 mL of diluted or undiluted sample into each well and incubate for 1.5 to 5 hours at RT OR overnight at 4°C. Aspirate samples from each well.
- Wash Buffer I Wash: Pipette 2 mL of 1X Wash Buffer I into each well and incubate for 5 minutes at RT. Repeat this 2 more times for a total of 3 washes using fresh buffer and aspirating out the buffer completely each time.
- Wash Buffer II Wash: Pipette 2 mL of 1X Wash Buffer II into each well and incubate for 5 minutes at RT. Repeat this 1 more time for a total of 2 washes using fresh buffer and aspirating out the buffer completely each time.
- Pipette 1 mL of the prepared Biotinylated Antibody Cocktail into the appropriate well and incubate for 1.5 to 2 hours at RT OR overnight at 4°C. Aspirate biotinylated antibody cocktail from each well.
- Wash membranes with Buffer I Wash followed by Buffer II Wash as directed previously.
- Pipette 2 mL of 1X HRP-Streptavidin into each well and incubate for 2 hours at RT OR overnight at 4°C. Aspirate HRP-Streptavidin from each well.
- Wash membranes with Buffer I Wash followed by Buffer II Wash as directed previously.
- Transfer the membranes to blotting paper, remove excess wash buffer and transfer print side up onto a plastic sheet.
- In a clean tube create a 1:1 Detection Buffer C and Detection Buffer D mix.

- Gently pipette 500 µL of the Detection Buffer mixture onto each membrane and incubate for 2 minutes at RT (DO NOT ROCK OR SHAKE).
- Carefully add another plastic sheet over the membrane to 'sandwich' the membrane.
- Transfer the sandwiched membranes to the chemiluminescence imaging system and expose.
- To store, tape the sheets together or use plastic wrap to secure them, and store at ≤ -20°C for future reference.

## 16. TROUBLESHOOTING

| Problem | Cause | Recommendation |
|---|---|---|
| No signals (not even the positive controls spots) | Chemiluminescent image is not working properly | Contact image manufacturer |
| | Too Short Exposure | Expose the membranes longer |
| | Degradation of components due to improper storage | Store entire kit at ≤ - 20°C. Do not use kit after expiration date. See storage guidelines. |
| | Improper preparation or dilution of the HRP-Streptavidin | Centrifuge vial briefly before use, mix well, and do not dilute more than 1000-fold |
| | Waiting too long before exposing | The entire detection process should be completed in 10-15 minutes |
| Positive controls spots signals visible but no other spots | Low sample protein levels | Decrease sample dilution, concentrate samples, or load more protein initially |
| | Skipped Sample Incubation Step | Samples must be loaded after the blocking step |
| | Too Short of Incubations | Ensure the incubations are performed for the appropriate time or try the optional overnight incubation(s) |
| Uneven signals and/or background | Bubbles present on or below membrane | Don't rock/rotate the tray too vigorously or pipette the sample or reagent with excessive force |
| | Insufficient sample or reagent volume | Load enough sample and reagent to completely cover the membrane |
| | Insufficient mixing of reagents | Gently mix all reagents before loading onto the membrane, especially the HRP-streptavidin and Biotin Antibody Cocktail |
| | Rocking/Rotating on an uneven surface while incubating | Rock/rotate on a flat surface or the sample or reagent can "pool" to one side |

| Problem | Cause | Recommendation |
|---|---|---|
| High background signals or all spots visible | Too much HRP-Streptavidin or Biotinylated Antibody Cocktail | Prepare these signal enhancing components precisely as instructed |
| | Membranes dried out | Do not let the membranes dry out during the experiment. Cover the incubation tray with the lid to minimize evaporation |
| | Sample Protein Concentration Too High | Increase dilution of the sample or load less protein |
| | Exposed Too Long | Decrease exposure time |
| | Insufficient Washing | Ensure all the wash steps are carried out and the wash buffer is removed completely after each wash step |
| | Non-specific binding | Ensure the blocking buffer is stored and used properly. |

## 17. NOTES

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