BioSentinel

505 S. Rosa Road, Suite 105
Madison, WI 53719
1-608-441-8174
Email: info@biosentinelpharma.com

BioSentinel Part No: L1006, Release Date: 02/11/2013

Matrix A Botulinum Neurotoxin Immunoprecipitation Kit Protocol

This protocol is intended for users who have purchased the Matrix A BoNT Immunoprecipitation Kit (product # A1014) as a standalone solution. Users who purchased the combination BoTest™ Matrix A BoNT Detection Kit (product # A1015) should refer to the protocol specific for that kit.

Matrix A BoNT Immunoprecipitation Kit 200 assays Cat. A1014

1.0 INTRODUCTION

The Matrix A Botulinum Neurotoxin (BoNT) Immunoprecipitation Kit is used to isolate BoNT serotype A (BoNT/A) from complex matrices such as serum, blood, food, water, bacterial supernatant, and pharmaceutical samples. The Matrix A Kit captures, concentrates, and isolates BoNT/A complex or holotoxin out of complex matrices from samples ranging in size from 100 μl to 50 ml. Upon isolation, captured BoNT/A can be analyzed by a user-chosen method. Depending on the matrix composition and sample size, femtomolar – picomolar BoNT/A concentrations can be isolated within 3 hours.

The Matrix A Beads consist of a proprietary anti-BoNT/A antibody covalently conjugated to magnetic beads. These beads are added to a sample containing BoNT/A and incubated to allow BoNT/A binding. Interfering compounds that might otherwise impede BoNT/A activity determinations are removed by washing the Matrix A Beads. The end-point analysis is not defined in this protocol and is dependent on the user's application. If an activity determination is required, the Matrix A Kit can be used in combination with the BoTest™ A/E BoNT Detection Kit.

2.0 DESCRIPTION

2.1 Materials Supplied

Matrix A™ BoNT/A Immunoprecipitation Kit (A1014)

Description	Composition	A1004	
		Size	Part #
Matrix A Beads	Magnetic beads covalently conjugated to chicken anti-BoNT/A antibody in PBS, 0.1% Tween-20, 0.05% Sodium Azide, 0.25% Casein, and 50% Glycerol	4 ml	A1012
10x Matrix Binding Buffer	500 mM Hepes-NaOH, pH 7.1, 250 mM NaCl, 1% Tween-20, 5% Casein, 0.05% Sodium Azide	5 ml	A1016
10x Matrix Wash Buffer	119 mM Phosphates, pH 7.4, 1370 mM NaCl, 27 mM KCl, 1% Tween-20	25 ml	A1013

2.2 Additional Required Materials

- Flat-bottom 96-well microtiter plates with covers
- Microtiter plate mixer
- Orbital shaker at room temperature or at 25 ºC if temperature control is available
- BoNT/A (optional, required for standardization and quantification purposes)
- 96-well magnetic separation plate compatible with chosen microtiter plates
- Plate washer or vacuum apparatus set up for washing magnetic beads (optional)
- Microcentrifuge (optional)
- High-quality (i.e. nanopure) H_2O

3.0 STORAGE

Description	Storage Temp.	Notes
Matrix A Beads	-20 ºC	Stable for a minimum of five days at 4 ºC upon removal from -20 ºC. DO NOT FREEZE AT -80 ºC.
10x Matrix Binding Buffer	-20 or -80 ºC	Stable for a minimum of five days at 4 ºC upon thawing.
10x Matrix Wash Buffer	-20 or -80 ºC	Stable for a minimum of five days at 4 ºC upon thawing.

4.0 SAFETY PRECAUTIONS

Normal precautions exercised in handling laboratory reagents should be followed.

5.0 GENERAL ASSAY CONSIDERATIONS

5.1 Required instrumentation and equipment

The Matrix A Kit uses antibody-conjugated magnetic beads (the Matrix A Beads) and requires a 96-well magnetic separation plate compatible with the microtiter plates being used. We recommend the 96-well magnetic plate from V&P Scientific (cat. #VP771H) used with black, flat-bottomed F96 MicroWell™ microtiter plates from NUNC (cat. #237105). Several other designs are available from various manufacturers that may provide satisfactory results although testing will be required.

Washing the Matrix A Beads can be performed by hand with a single- or multi-channel pipette. However, assay times can be reduced and throughput increased by using an automatic plate washer or vacuum apparatus configured for magnetic beads. The washer or vacuum should be tested and adjusted to minimize bead loss during washing. Refer to the instrument's manual for proper settings and testing protocols.

Assay performance is highly dependent on thorough resuspension of the Matrix Beads where specified in the protocol and then maintaining the suspension during all incubation steps. A microplate mixer can be used to thoroughly resuspend the Matrix Beads after pelleting. Bead suspensions can then be maintained using an orbital shaker or an incubating microtiter plate shaker during incubation periods.

5.2. Buffer considerations

The Matrix A Kit comes with a 10x Matrix Binding Buffer that is added to samples in order to adjust buffer conditions and reduce non-specific binding to the beads. In cases where maximum sample volume is critical, this buffer may be excluded although exclusion may result in poor assay performance.

The protocol below may need to be modified to account for sample viscosity, non-specific binding, or other factors, depending on the nature of the BoNT/A-containing sample. Additional sample treatments or bead washing steps may be required to optimize assay performance (see 6.0 ADDITIONAL INFORMATION).

5.3 Matrix A Bead use consideration

Assay performance is critically dependent on executing washing and supernatant removal steps without accidental removal or loss of the beads. The recommended magnetic separation times should not be reduced without thorough testing in order to minimize bead loss. In addition, separation times may need to be increased if magnets other than the recommended magnets are used (see 5.1).

The microtiter plate should be placed on the magnetic 96-well separator as soon as possible after completion of mixing and incubation periods as noted in 7.0 BASIC ASSAY PROTOCOL. This prevents the beads from settling to the bottom of the wells before separation stesp and ensures complete bead sequestering to the sides of the wells, limiting accidental aspiration of beads from the well bottom during supernatant removal. When removing supernatants manually, insert the pipette tip in the center of the well and slowly remove the liquid.

6.0 ADDITIONAL INFORMATION

An applications guide can be found at www.biosentinelpharma.com.

7.0 BASIC ASSAY PROTOCOL

Part 1. Reagent preparation.

1. Thaw the 10x Matrix Wash and 10x Matrix Binding Buffers at room temperature.
    a. Allow the buffers to warm completely to room temperature.
    b. 10x Matrix Binding Buffer will have a cloudy appearance.
    c. Vortex buffers for 5 seconds to mix.
2. Calculate the number of wells required.
    a. At least one well is required for each unknown, control, and standard to be assayed.
    b. It is recommended that each sample (unknown, control, and standard) be assayed in duplicate or triplicate, increasing the number of wells used to 2 or 3 wells per sample.
    c. Include one or more control wells containing no BoNT/A, ideally using samples containing the same matrix or buffers as the unknown samples.
    d. For quantitative assays, it is recommended that a standard dilution series of known BoNT/A quantities be prepared. A range of 0.1 pM to 1 nM BoNT is recommended.
    e. The calculated number of wells will determine the volumes of buffers to be prepared in the following steps. **It is recommended that the calculated number of wells be increased 10 – 25% to account for pipetting errors and loss during dispensing.**
3. Prepare 1 ml of 1x Matrix Wash Buffer for every test well.
    a. Prepare 1x Matrix Wash Buffer by diluting 1 part 10x BoTest™ Matrix A Wash Buffer with 9 parts H_2O and mixing well by inversion.
    b. Keep 1x Matrix Wash Buffer at room temperature until used.

Part 2. BoNT/A binding and isolation.

4. Warm the Matrix A Beads for 15 minutes at room temperature.
5. Prepare samples including unknowns, controls, and standards.
    a. Sample volumes of 100 to 225 µl per well can be tested using the microtiter plate format. For smaller volumes, increase the sample volume to at least 100 µl using a buffer compatible with your samples (e.g. PBS or the matrix being tested). It is recommended that all samples have the same volume.
    b. (Optional) For samples containing particulate matter, a pre-clearing step is recommended to remove solid material from the sample. Spin samples in a microcentrifuge for >10 minutes at >12,000 x *g* and carefully collect the supernatants for testing.
    c. For sample volumes > 225 µl, please consult the application guide (see 6.0 ADDITIONAL INFORMATION).
6. Dispense the appropriate volume of unknowns, controls, and standards into wells.
7. Dispense a volume of 10x Matrix Binding Buffer to each well equal to one tenth ($1/10^{th}$) of the sample volume.
    a. Example: If 100 µl of the sample was dispensed into a well, add 10 µl of 10x Matrix Binding Buffer to the sample in the well.
8. Thoroughly resuspend the Matrix A Beads by vigorously vortexing for 5 seconds.

   a. The beads will settle to the bottom of the stock tube over time and must be thoroughly resuspended before removal. Visually verify that the beads are in a homogenous suspension and, if needed, repeat vortexing.

9. **Immediately** dispense 20 µl Matrix A Beads into each well containing sample.
   a. Maintain a homogenous bead stock suspension during dispensing. If needed, vortex the bead stock again for 5 seconds.

10. Cover the plate and incubate at 25 °C or room temperature with shaking for 2 hours.
    a. The beads must be maintained in suspension for optimal assay performance. Mix plate for 10 seconds using a microtiter plate mixer before transferring to an orbital shaker.

11. Wash the Matrix Beads with 1x Matrix Wash Buffer.
    a. Remove the plate from the orbital shaker and **immediately** separate beads for ≥ 2 minutes using a 96-well magnetic separation plate.
    b. Using a pipette tip, gently remove the supernatants being careful not to remove any beads.
    c. Remove the plate from the magnetic separator and add 200 µl 1x BoTest™ Matrix A **Wash Buffer**.
    d. Mix plate for 10 seconds using a 96-well plate mixer. After mixing, **immediately** separate beads for 2 minutes using a 96-well magnetic separation plate.
    e. Repeat Steps (b) through (d) twice for a total of 3 washes. Additional wash steps may be required for high viscosity samples or samples containing particulate matter.

12. The BoNT/A bound to the Matrix Beads is now ready for user-defined analysis.