

# **128-Plex STANDARD BARCODED MAGNETIC BEADS (BMBs)**

*For Research Use Only*

## **Product Description**

The Standard 128-plex Barcoded Magnetic Beads (BMBs) are intended for absorption of antibody/protein for multiplex bioassay for protein based test. The BMB, utilizing a **digital technology** instead of conventional analog methodology, offers unmatched decoding accuracy, excellent fluorescence detection precision; and 128 numbers of barcodes for multiplex tests.

## **Specifications**

Package volume	2 mL
Dispersion media	Storage Buffer
Number of BMBs	Approx. 50,000
BMB Size	72 $\mu\text{m}$ x 27 $\mu\text{m}$ x 5 $\mu\text{m}$

## **Product Storage**

Store at Room Temperature (15-30°C).

The Standard BMBs are stable and good for antibody/protein adsorption for up to two years or the labeled expiration date when properly stored. Once BMBs have been coated, they should be stored at 2-8°C. Stability may vary for each of the coupled product.

## **Warning and Precaution**

Always ensure that BMBs are homogeneously resuspended prior to dispensing.

## **Test Principle, Summary and Explanation**

The Applied BioCode System is a flexible multiplexing platform for detecting and analyzing analytes using the BMB. The immunoassays are performed in an aqueous, solid-phase format, both quickly and efficiently. The BioCode Analyzer and BMB technology offers multiplex capability for simultaneous detection of up to 128 different analytes within a single sample.

With BMBs technology, for immunoassay, each BMB type is analogous to an individually coated well in a conventional ELISA assay providing a capture surface for a specific analyte. The capture antibodies are adsorbed to the Standard BMBs. After incubation with analytes and biotin labeled detection antibodies, the SA-PE is added for analytes detection. The BioCode Analyzer completes the data collection and reports the results in different formats.

## **Reagents and Materials Supplied**

Standard BMBs (Part No. 44-B0101-0XXX)

**Note:** "0XXX" represents the Barcode number from 0 to 127. The last three digits will identify the bar code e.g. 0115 = bar code 115.

## **Reagents and Materials required but not provided**

### **Materials:**

BioCode 1000A Analyzer System (Part# 41-A0002)

Magnetic Microplate Separator (Part# 01-M0001)

Magnetic stand for Eppendorf tubes

Pipettes and pipette tips

1.5 mL and 2 mL nuclease-free microcentrifuge tubes and racks or equivalent

Vortexer

Microcentrifuge with rotor for 1.5 mL microcentrifuge tubes or equivalent

96-well Incubator/Shaker, Vortemp 56 or equivalent

96-well, flat-bottomed, microtiter plates (Thermo Scientific Part#9205 or VWR Part#62402-933 or equivalent).

Shaker, Bioshake XP or equivalent.

**Reagents:**

Coating Buffer (contains no amino groups, e.g. PBS or MES or Carbonate with pH 6.0 – 9.6)  
 SA-PE conjugates (Invitrogen)  
 IX PBS Buffer, pH 7.4  
 Wash Buffer: 0.05% Tween-20 in PBS. (PBST)  
 Block Buffer: 1% BSA in PBS  
 Assay Buffer: 0.05% Tween-20, 1% BSA in PBS  
 Standard Barcoded Magnetic Beads  
 Detection Buffer (Applied BioCode, Inc.)  
 Antispecies Antibody PE Conjugates (Jackson Immuno Research Lab or equivalent)  
 Detection Antibody (Biotinylated).

**Note:**

Coating antibody/protein by adsorption can be performed overnight at different pH  $\geq 6.0$  (pH less than 6.0 will cause the beads to be bleached out) or in a different buffer. If your antibody/protein does not coat satisfactorily under these recommendations, try an alternate coating buffer, and pH.

Researchers are advised to optimize the use of BMBs in any application.

**Absorption of Capture Antibody to Standard BMBs:**

1. Place 5000-50,000 Standard BMBs in a 2 mL micro centrifuge tube. Quick spin 10 seconds. Put the tube on magnetic stand, and carefully remove the supernatant.
2. Wash the BMBs twice with 500  $\mu$ L Coating Buffer with pH 6.0 - 9.6) by vortex 10 seconds, and quick spin for 10 seconds. Use magnetic stand to hold the beads, and remove the supernatant.
3. Prepare capture antibody in Coating Buffer (0.1-5  $\mu$ g/mL depending on antibodies. Note: It is important to have the antibody free of other proteins or amine-based salts that can interfere with the antibody adsorption).
4. Add 500  $\mu$ L diluted capture antibody to BMBs, vortex 5 second, and quick spin for 5 seconds.
5. Incubate the BMBs overnight (16-22 hours) at room temperature with shaking 1400 – 1500 rpm (e.g. Bio Shaker XP).
6. Wash the BMBs twice with 500  $\mu$ L PBST, and once with 500  $\mu$ L PBS/1% BSA by vortex 10 seconds, and quick spin for 10 seconds. Use magnetic stand to hold the beads, and remove the supernatant.
7. Block the BMBs in 500  $\mu$ L PBS/1% BSA for 1 hour incubation at RT with shaking.
8. Wash the BMBs once with 500  $\mu$ L PBST, and once with 500  $\mu$ L Assay Buffer, and store the BMBs in Assay Buffer with 0.1%  $\text{NaN}_3$  (or 0.1% ProClin) at 4°C or do the immunoassay immediately.

**1. Protocol for Immuno-Detection of Coated Antibody:**

2. Take the volume containing the number of BMBs necessary for the experiment (e.g. 100 bead/well, for 8 wells), and add into a new tube.
3. Remove supernatant, and add 450  $\mu$ L Assay Buffer to the BMBs, and aliquot 50  $\mu$ L per well into 8 wells.
4. Dilute Antispecies Antibody PE (Jackson ImmunoResearch Lab.) in 200  $\mu$ L Assay Buffer to make 5120 ng/mL solution, and make 5 times 4-fold serial dilution for: 1280 ng/mL, 320 ng/mL, 80 ng/mL, 20 ng/mL, 5 ng/mL, 1.25 ng/mL, and 0 ng/mL).
5. Add 50  $\mu$ L diluted sample to dispensed BMBs.
6. Incubate sample at RT for 30 min with shaking at 600-700rpm (Incubator-Shaker Vortemp 56).
7. Wash the BMBs 1~2X with 120  $\mu$ L Wash Buffer with 5-10min shaking at 700rpm.
8. Spread BMBs in 190  $\mu$ L Detection Buffer, and scan with BioCode 1000A Analyzer.

**2. Protocol for Immuno assay for Analyte:**

1. Plan ahead to determine the number of sample to be run in duplicate. Take the volume containing the number of BMBs necessary for the experiment, and add into a new tube.

2. Remove supernatant, and add Assay Buffer to the BMBs (number of sample x 25  $\mu$ L), and aliquot 25  $\mu$ L per well. [If working with 96well filter plate, pre-wet the well with 100  $\mu$ L Wash Buffer (remove buffer using vacuum), and 100  $\mu$ L Assay Buffer, and then aliquot 25  $\mu$ L beads into the well].
3. Dilute Analyte in Assay Buffer and add 50  $\mu$ L diluted Analyte to BMBs.
4. Incubate sample at RT for 30 min with shaking at 600-700 rpm.
5. Add 25  $\mu$ L Detection Antibody (Biotinylated Antibody, 0.1-0.5  $\mu$ g/mL: needs to be optimized for each antibody) per well.
6. Incubate at RT for 0.5 - 2 hours with 600-700 rpm shaking.
7. Wash the BMBs with 120  $\mu$ L Wash Buffer with 5-10 min shaking at 600-700 rpm. Remove supernatant.
8. Dilute Streptavidin-R-PE (1 mg/mL) to a concentration of 5  $\mu$ g/mL, add 50  $\mu$ L per well. Incubate at RT for 15-30 min with 600-700 rpm shaking.
9. Wash samples twice with 120  $\mu$ L Wash Buffer with 5-10 min shaking at 600-700 rpm. (If working with 96 well filter plate, transfer BMBs into 96-well flat-bottomed plate in Wash Buffer).
10. Re-suspend BMBs in 200  $\mu$ L Detection Buffer, and scan using BioCode 1000A Analyzer.

**Note:** The last wash step can be done using Detection Buffer if background signal is high.

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