128-Plex CARBOXYL BARCODED MAGNETIC BEADS (BMBs)

For Research Use Only

Product Description

The 128-plex Barcoded Magnetic Bead (BMB) technology was developed for multiplex bioassay for DNA/RNA and 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 µm x 27 µm x 5 µm

Product Storage

Store at Room Temperature (15-30°C).
The carboxyl BMBs are stable and good for probes coupling for up to two years or the labeled expiration date when properly stored. Once BMBs have been coupled 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 targets using the BMB. A wide variety of assay types, such as DNA hybridization assays, and immunoassays are performed in an aqueous, homogeneous 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, molecular reactions take place on the surface of BMBs. For each samples, target-specific capture probes are covalently linked to a specific set of BMBs. Biotins labeled targets are captured by the bead-bound capture probes in a hybridization suspension. Finally, the Streptavidin--R-Phycoerythrin Conjugate (SA-PE) will be added to the samples for hybridization of targets and detection. 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 covalently linked to the carboxyl BMBs. After incubation with analyte and biotin labeled detection antibodies, the SA-PE is added for analyte detection. The BioCode Software completes the collect data and reports the results in a matter of seconds.

Reagents and Materials Supplied

Carboxyl BMBs (Part No. 44-B0102-0XXX)
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)
Pipettes and pipette tips
1.5 mL and 2 mL nuclease-free microcentrifuge tubes and racks
Vortexer
Microcentrifuge with rotor for 1.5 mL microcentrifuge tubes
Heated Incubator/Shaker set at 52°C
96-well, flat-bottomed, microtiter plates (Thermo Scientific Part#9205 or VWR Part#62402-933).
Bioshake XP

Reagents:

Detection buffer (Part# 44-D0001)
25 mM MES Buffer (pH 6.0)
25 mM MES-T Buffer (pH6.0), 0.01% Tween 20 added.
Tris-HCl 50 mM, pH 7.4
1x PBS (pH 7.4); 1xPBS/1% BSA
1x PBS-T (Tween-20, 0.1%)
Nuclease-free H_2O
Capture Oligo: 100 µM in H_2O (e.g. 5' aminoC6 modified (NH2))
Analyte: Biotin labeled target oligo, or amplicon
Streptavidin-R-Phycoerythrin Conjugate (SA-PE) (1 mg/mL)
Hybridization buffer : TMAC Buffer (TMAC 3M, Tris-HCl 50mM, pH 8.0, Sarkosyl 0.1%, EDTA 4mM), or 5x SSC, 0.1% SDS
EDC

Note:

Coupling can be performed at different coupling pH or in a different buffer. If your oligo probe or protein does not couple satisfactorily under these recommendations, try an alternate coupling buffer, and pH. Researchers are advised to optimize the use of BMBs in any application.

Procedure I:

Single Step Coupling of AminoC6 Modified Oligo Probe on Carboxyl BMBs

Capture oligo coupling to BMBs:

This coupling procedure is recommended for coupling up to 500,000 BMBs within a 2 mL mircofuge tube.

1. Vortex BMB tube for 10-15 seconds, quick spin, place each tube on magnetic stand for 20-30 seconds, and carefully remove the supernatant.
2. Wash the BMBs 2X with 500 µl of MES-T buffer repeating step 1 in between each wash.
3. Add 159 µl MES-T buffer to the BMBs, vortex 5 second.
4. Add 1 µl Capture oligo (e.g.5' AminoC6 modified oligo: 100 pM/µl in H_2O), vortex 5 second.
5. Freshly preparing EDC 10 mg/mL solution (10 mg EDC in 1 mL of cold MES buffer),
6. Immediately add 40 µl of fresh prepared EDC solution (200 µl final), vortex for 5 second, then incubate for at least 2 hrs at RT, with shaking at 1600 rpm (BioShake XP).
7. Remove the supernatant as in step 1, and treat the BMBs with 500 µl Tris-HCl 50 mM, pH 7.4 for 15 min at RT, with shaking at 1600 rpm.
8. Wash the BMBs once with 500 µl PBS/1% BSA.
9. Block samples with 500 µl PBS/1% BSA, incubate for 1 hr at RT, with shaking at 1600 rpm.

10. Remove the blocking buffer. Resuspend the beads in 500 µl PBS-T. (Note: antimicrobial reagent can be added e.g. 0.1% Proclin). Count the beads, and store the beads at 2-8°C, or process for the hybridization.

Procedure II:

Hybridization using 96 well flat bottomed plates

1. Place tube containing coupled BMB onto the magnetic stand and remove PBS-T Supernatant buffer.
2. Add to the above tube TMAC hybridization buffer so that the total amount is equal to the number of wells desired (50 µl/well) and vortex.
3. Mix tube vigorously and pipette at least 5 times before dispensing 45 µl into each well of the plate, making sure to repeat vigorous mixing to ensure even bead distribution into each well.
4. Add 5 µl analyte (Diluted standard samples, asymmetric PCR amplicon, or denatured PCR amplicon etc.) into corresponding well, and mix by pipetting up and down 3 times. Incubate for **10-15 min** at 52°C with shaking at 700 rpm (Labnet Vortemp 56).

Substrate Treatment:

5. Prepare Strepdavidin-PE: 30 µg/mL in PBS-T buffer.
6. Add 10 µl SA-PE solution per well, mix by pipetting up and down 3 times, and continue shaking at 700 rpm for **5 min at 52°C.**
7. Place the plate on a strong magnetic micro plate separator to hold back the beads, and remove supernatant using wide orifice tips.
8. Wash the beads 1X-2X by adding 180 µl PBS-T buffer per well, mix by pipetting up and down 3 times, and remove supernatant using wide orifice tips, and the strong magnetic plate separator.
9. Add 200 µl Detection buffer into each well by pipetting up and down 10 times carefully to avoid forming air bubbles in the solution. (Remove any bubbles if necessary by blowing with 1 mL pipette to break bubbles).
10. Seal the plate with a clear Plate Seal if necessary, and scan it with BioCode 1000A BMBs Analyzer.

Procedure III (recommended procedure):

Antigen Detection; 2-Step sulfo-NHS/EDC Coupling of Capture Antibody

Materials:

Capture Antibody
Biotin Labeled Detection Antibody
Standard Analyte
PBS: Dilute 10xPBS to 1xPBS, pH 7.4 in nuclease-free water.
EDC: 50 mg in 1 mL cold MES buffer, prepared freshly
Sulfo - NHS: 50 mg in 1 mL MES buffer, prepared freshly
Activating Buffer: MES 50 mM pH 6.3; Coupling Buffer: MES 25 mM, pH 6.0
NaCl-T: 1 M NaCl, 0.01% Tween 20
Wash Buffer: 0.1% Tween-20 in PBS.
Block Buffer: 1% BSA in PBS
SA-PE: Streptavidin-R-Phycoerythrin Conjugate (1mg/ml)-Invitrogen
Assay Buffer: 0.1% Tween-20, 1% BSA, 0.1% Proclin in PBS

Antibody Coupling:

1. Wash 5000-50,000 Carboxyl BMB twice with 500 µl MES buffer (25 mM, pH 4.0-6.0): vortex BMBs for 10 seconds, quick spin, hold BMBs with magnetic stand. Discard the residual buffer. Resuspend BMBs in 380 µl MES buffer.
2. **Activate the BMBs:**
   Add 70 µl Sulfo-NHS 50 mg/mL in MES buffer, vortex 5 second. Add 50 µl EDC 50 mg/mL in cold MES buffer, vortex 5 second. Incubate 30 min at RT with shacking at 1600 rpm.

Antibody coupling:

Wash BMBs twice with 500 µl MES buffer. Dilute capture antibody with MES buffer to a concentration of approx. 25-200 µg/mL, mix 450 µl with BMBs, and incubate two hours at RT with shaking at 1600 rpm.

3. Remove supernatant. Wash BMBs once with 500 µl PBST, and once with 500 µl NaCl-Tween buffer.
4. Incubate the BMBs in 500 µl NaCl-Tween buffer for one hour at RT with shaking at 1600 rpm.
5. Wash BMBs once with 500 µl PBS/1%BSA (block buffer). Block the non specific sites with 500 µl of block buffer to the BMBs. Incubate for at least 1 hour at RT with 1600 rpm. Wash the BMBs once with 500 µl Assay buffer.
6. Store the antibody coupled BMBs in 500 µl Assay buffer at 4°C.

Antigen Detection:

1. For each dilution sample, dispense 20 µl Assay buffer, and 5 µl BMBs into each well of the 96-well sample plate.
2. Dilute standard antigen from 5000 pg/mL to zero in Assay buffer. (5120 pg/mL, 2560 pg/mL, 1280 pg/mL, 640 pg/L, 320 pg/mL, 160 pg/mL, 80 pg/mL, and 0 pg/mL). Add 50 µl diluted standard sample to the wells containing dispensed BMBs.
3. Incubate at RT for 30 minutes with shaking at 700rpm.
4. Dilute biotinylated detection antibody to an appropriate concentration in Assay buffer (0.05-0.5 µg/mL). Add 25 µl per sample. Continue incubate at RT for 2 hours with shaking at 700rpm.
5. Remove the supernatant using wide orifice tips, with strong magnetic micro plate separator to hold the BMBs to the bottom of the plate. Wash samples once with 120 µl wash buffer by shaking at RT for 10 min at 700 rpm.
6. Dilute Streptavidin-R-PE (1 mg/mL) to a concentration of 5 µg/mL in assay buffer. Add 50 µl per sample. Incubate at RT for 30 min with shaking at 700rpm.
7. Wash samples 1X with 120 µl wash buffer by shaking at RT for 10 min at 700 rpm.
8. If necessary, wash samples 1X with 120 µl Detection buffer by shaking at RT for 10 min at 700 rpm.
9. Mix the BMBs with 200 µl Detection buffer (Pipetting up-down more than 10 times)
10. Seal the plate with a clear Plate Seal if necessary, and scan in BioCode 1000A Analyzer.

References:

Nakajima N. and Ikade Y. (1995). Mechanism of Amide Formation by Carbodiimide for Bioconjugation in Aqueous Media. *Bioconjugate Chem.* 6(1): 123-130.

Sehgal D. and Vijay I.K. (1994). A method for the high efficiency of water-soluble carbodiimide-mediated amidation, *Anal Biochem.* 218 (1): 87-91.

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