PACKAGE INSERT
Applied BioCode, Inc. - Beads

For Coupling Nucleic Acids:

CARBOXYL BARCODED MAGNETIC BEADS (BMBs)
128-Plex - Part Number 44-B0102
4096-Plex - Part Number 44-B0302

OR

For Coupling Proteins:

P-CARBOXYL BARCODED MAGNETIC BEADS (BMBs)
128-Plex - Part Number 44-B0112
4096-Plex - Part Number 44-B0312

For Research Use Only

Product Description

The 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 or 4096 numbers of barcodes for multiplex tests.

Carboxyl or P-Carboxyl are ordered either as regular Carboxyl beads for coupling DNA Capture probes or as P-Carboxyl beads ideally suited for coupling Proteins. Note: ***"XXXX" represents the Barcode numbers from either 0 to 127 for the 128-plex beads or 0 to 4095 for the 4096-plex beads.***

Specifications

Package volume	1.5 mL package in a 2 mL tube
Dispersion media	Storage Buffer
Number of BMBs	50,000 for standard packaging (custom packaging available upon request)

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

1. Avoid exposure of the BMBs to buffers with pH less than five for extended periods (less than 12 hours) as this may cause bleaching or fading of the bar code elements.
2. Always ensure that BMBs are homogeneously suspended 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 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 compiles the collect data and reports the result in a matter of seconds.

Note: The following equipment, materials, reagents and procedures are suggestions for preparing and using the Carboxyl Barcoded Magnetic Beads (BMBs). Optimization of coupling conditions, incubation/hybridization as well as target interactions for multiplexing will likely be required.

Equipment and Materials

- BioCode 1000A analyzer system (Part# 41-A0002) for 128-plex or a BioCode 2000 analyzer system (Part# 41-A0004) for both 128 and 4096 plex beads.
- Magnetic micro-plate separator (Part# 01-M0001)
- Clear micro-plate seals (recommended)
- Pipettes and pipette tips
- 1.5-mL and 2-mL nuclease-free micro-centrifuge tubes and racks
- Vortexer
- Microcentrifuge with rotor for 1.5-mL micro-centrifuge tubes
- Heated Incubator/Shaker for hybridization/incubation (BioShake XP) set at 52°C for DNA and room temperature for proteins.
- Tube or plate shaker for coupling (BioShake XP)
- 96-well, flat-bottomed, micro titer plates (Greiner Bio-One Part# 655101).
- Magnet rack for micro centrifuge tubes

Procedure I:

Single Step Coupling of AminoC6 Modified Oligo Probe on Carboxyl BMBs

- 50 mM MES Buffer (pH 5.0)
- 50 mM MES-T Buffer (pH 5.0), 0.01% Tween 20.
- 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 (e.g. 5' aminoC6 modified (NH2)) in H_2O or in 0.1x TE buffer (1mM Tris-HCl, 0.1mM EDTA, pH 7.5)
- Analyte: Biotin labeled target oligo, or amplicon
- Streptavidin-R-Phycoerythrin Conjugate (SA-PE) (1 mg/mL)
- Hybridization buffer : TMAC Buffer (TMAC 3M, Tris-HCl 50 mM, pH 8.0, Sarkosyl 0.1%, EDTA 4 mM). EDC 10 mg/mL of EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) in cold Activation Buffer (prepare fresh).
  Note: EDC is moisture sensitive. It's better to use single-use packaged reagent.
- Detection Buffer (Part# 44-D0002)

I. Capture oligo coupling to BMBs

This coupling procedure is recommended for coupling up to 500,000 BMBs within a V-bottomed 2-mL micro-centrifuge 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 twice with 200 µL of MES-T buffer repeating step 1 in between each wash.
3. Add 159 µL MES-T buffer to the BMBs, and do sonication for 20".
4. Add 1 µL Capture oligo, vortex 5 second.
5. Quick spin, incubate 5-10 min with shaking at 1600 rpm
6. Freshly preparing EDC 10 mg/mL solution (10 mg EDC in 1 mL of cold MES buffer),
7. Immediately add 40 µL of fresh prepared EDC solution (200 µL final), vortex for 5 second, then incubate for at least 2 hours at room temperature, with shaking at 1600 rpm (BioShake XP).
8. Option: Add 10µL fresh prepared EDC solution after 1 hour shaking
9. Remove the supernatant as in step 1, and treat the BMBs with 500 µL Tris-HCl 50 mM, pH 7.4 for 15 minutes at room temperature, with shaking at 1600 rpm.
10. Wash the BMBs once with 500 µL PBS/1% BSA.
11. Block samples with 500 µL PBS/1% BSA, incubate for 1 hour at room temperature, with shaking at 1600 rpm.
12. Remove the supernatant. Wash BMBs twice with 500µL PBST buffer.
13. Re-suspend the BMBs in 100-1000 µL PBS-T. (Note: antimicrobial reagent can be added e.g. 0.1% Proclin-950).
14. Store the BMBs at 2-8°C, or process for the hybridization.

II. Hybridization using 96-well, flat bottomed, micro titer plates

1. Take the volume of beads necessary for hybridization experiment into a new 5ml tube (50-100 BMBs per target and per well).
2. Place tube containing coupled BMB onto the magnetic stand and remove PBS-T buffer.
3. 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.
4. 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.
5. 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**-**30 minutes** at 52°C with shaking at 700 rpm (Labnet Vortemp 56).

III. SA-PE Treatment

1. Place the plate on the magnetic micro-plate separator, and remove the supernatant.
2. Prepare Strepdavidin-PE solution: 2.5 µg/mL in Hybridization buffer.
3. Add 50 µL SA-PE solution per well, and continue shaking at 700 rpm for **5-15 minutes at 52°C.**
4. Wash the plate twice with PBS-T using BioTek washer (ELx50 Model 8M, or equivalent). If necessary, place the plate on a magnetic micro-plate separator to hold back the BMBs, and remove the remaining supernatant.
5. Note: If automatic washer is not available, manual wash the BMBs twice by adding 180 µL PBS-T buffer per well, mix by pipetting up and down 3 times, and remove supernatant using magnetic micro-plate separator.
6. 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).
7. Seal the plate with a clear Plate Seal if necessary, and scan it with BioCode 1000A BMBs Analyzer.

Procedure II - Protocol for Protein Coupling on BMBs

The following is a suggested protocol for conjugating and performing the immunoassay using Magnetic Barcoded Beads (BMB). This protocol was developed for coupling TNF-α antibody using a two step Sulfo/EDC coupling. Because of variations in antibody/antigen targets, pi (isoelectric point), concentrations and other characteristics, protocol optimization will likely be required and the following is suggested only as a starting point for your assay development.

Reagents for Proteins:

- Activation Buffer: 100mM MES (2-(N-morpholino) ethanesulfonic acid), 15% ethanol, adjusted for pH 6.0.
- Coupling Buffer: 100mM MES, 140 mM guanidineHCl, adjusted for pH 5.0.
  Note: Use the coupling buffer with pH that is slightly below the protein/antibody pi.
- Sulfo-NHS: 50 mg/mL of Sulfo-NHS (*N* –Hydroxysulfosuccinimide) in cold Activation Buffer (prepare fresh).
- EDC: 50mg/mL of EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) in cold Activation Buffer (prepare fresh).
  Note: Sulfo-NHS & EDC is moisture sensitive. It's better to use single-use packaged reagent.
- PBS: Phosphate Buffered Saline solution pH 7.4.
- PBS-T: Phosphate Buffered Saline-Tween: 0.05% Tween-20 in PBS.
- NaCl-Tween: 0.01% Tween-20 in 1M NaCl.
- Tris-HCl Buffer: 50mM Tris-HCl, pH7.5.
- Blocking Buffer: 1% BSA (Bovine Serum Albumin) with 1% NFDM (non-fat dried milk) in PBS.
- Assay Buffer: 1% BSA in PBS-T with 0.05% ProClin 950
- SA-PE: Streptavidin-Phycoerythrin (1.0mg/mL of SA-PE stock solution) diluted in Assay Buffer to 2µg/mL.
- Detection Buffer: ABC P/N 44-D0002 (500mL)
- Assay specific Capture antibody, antigen with biotinylated Detection Antibody.

I. Couple Antibody or Antigen onto BMBs in 2mL Tube

Recommended number of beads: up to 100,000
Note: The capture antigen/antibody should be suspended in a non-amine buffer (e.g. PBS)

1. Transfer up to 100k BMB into a labeled V-bottomed 2mL micro-centrifuge tube. Vortex BMB tube for 10-15 seconds, quick spin, place each tube on magnetic stand for 20-30 seconds.
2. Remove supernatant and wash twice with 100µL Activation Buffer as step 1.
3. Remove supernatant and add 76µL Activation buffer. Option: Sonication for 20 seconds.
4. Add 14µL fresh prepared 50mg/mL Sulfo-NHS to beads. Vortex 5 seconds.
5. Add 10µL fresh prepared 50mg/mL EDC to beads (vortex 5sec). Vortex 5 seconds, and quick spin down.
6. Incubate on shaker/mixer at Room Temperature for 20-30 min. *Note: for BioShaker XP, run at 1600rpm.*
7. Remove supernatant and wash twice with 100µL Coupling Buffer as step 1.
8. Remove supernatant and add 100µL Ag or Ab (Diluted with Coupling Buffer). Vortex 5 seconds and quick spin.

Note: If coupling Antigen (Ag) to bead, we suggest you start with 40µg/mL. If coupling with Antibody (Ab), start with 150µg/mL.

9. Incubate on shaker/mixer at Room Temperature for 2 hrs.
10. Remove supernatant and wash once with 500µL NaCl-Tween buffer as step 1.
11. Remove supernatant and add 500µL PBST
12. Incubate on mixer at Room Temperature for 15 min
13. Remove supernatant and add 500µL, 50mM TRIS-HCL pH 7.5 Buffer
14. Incubate on mixer at Room Temperature for 30 min
15. Remove supernatant and add 500µL, 50mM TRIS-HCL pH 7.5 Buffer
16. Incubate on mixer at Room Temperature for 10 min
17. Remove supernatant and add 500µL NaCl-Tween buffer
18. Incubate on mixer at Room Temperature for 5 min
19. Remove supernatant and wash once with 500µL of Blocking Buffer (PBS/1% BSA, 1% NFDM).
20. Remove supernatant and add 500µL of Blocking Buffer.
21. Incubate on mixer at Room Temperature for 30-60 min
22. Remove supernatant and wash twice with500µL Assay buffer (1% BSA in PBS-T).
23. Re-suspend the BMBs in 100-1000µL Assay buffer, and store the beads at 2-8°C, or perform the immunoassay immediately).

II. Add Antigen (or Antibody) Sample in 96-well Plate

1. Pre-load the 96-well flat bottomed assay plate by liquating 25µL of Assay buffer into each well.
2. Pool the BMBs necessary for the assay. Place tube containing coupled BMB onto the magnetic stand and remove the supernatant.
3. Add to the above tube the assay buffer so that the total amount is equal to the number of wells desired (50 µL/well), plus 5% extra.
4. Vortex the BMBs vigorously before dispensing them into the wells of the 96-Well plate. A Mix BMBs by pipetting several times between dispenses. *Note: target at least 100 BMBs per well.*
5. Incubate the plate 5 minutes at room temperature with shaking at 650-800 rpm.
6. Place the plate onto a strong magnetic micro plate separator to hold the BMBs and slowly remove the supernatant.
   Note: If a filter plate is used, remove supernatant with Vacuum Manifold.
7. Remove the plate from magnetic.
8. Add 50µL of diluted Sample (diluted with Assay Buffer), and/or Standard to each targeted well.
9. Incubate in a shaker at 650-800 rpm, at room temperature for 1-2 Hrs.
10. Wash twice with PBS-T buffer using BioTek washer (ELx50 Model 8M, or equivalent), or manual wash the BMBs twice by adding 150 µL PBS-T buffer per well, and incubate at 650 rpm, room temperature for 5 minutes.

III. Add Secondary Antibody

1. Remove supernatant using the magnetic plate separator and add 50µL biotinylated detection Ab (diluted with Assay buffer).
2. Incubate at 650-800 rpm, Room Temperature for 30-60 minutes.
3. Wash the plate twice with PBS-T using BioTek washer (ELx50 Model 8M, or equivalent). Remove supernatant using the magnetic plate separator and wash twice with PBST at 700-1000 rpm, RT for 5 minutes.

IV. Add SA-PE:

1. Prepare 5µg/ml SA-PE solution (diluted with assay buffer). Note approximately 5mL for one 96 well plate.
2. Remove the supernatant a strong magnetic micro plate separator and add 50µl SA-PE solution to each well.
3. Incubate at 650-800 rpm, Room Temperature for 20-30 minutes in the dark.
4. Wash the plate twice with PBS-T using BioTek washer (ELx50 Model 8M, or equivalent), or manually wash four times with 150µlL PBS-T using the magnetic plate separator.
5. Remove the supernatant using the magnetic plate separator and add 200µL Detection Buffer. Mix the BMB by pipetting up-down several times, and blow out any bubbles before scanning.
6. *Notes: If filter plate was used for immunoassay, transfer the BMBs in Detection buffer to a flat bottomed 96-well plate.*
7. Seal the plate, and place it in BioCode Analyzer for decoding and fluorescence detection.

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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