Applied BioCode

24-Plex Carboxyl Barcoded Magnetic Beads Protein Coupling Kit

Protein/Antibody Coupling and Immunoassay Instructions

Part Number: 64-R0112-PIN

For Exclusive Use with
Applied BioCode 1000A or 2000 Analyzer

For Research Use Only - Not for Use in Diagnostic Procedures

INTRODUCTIONS

Intended Use

The 24-plex Carboxyl Barcoded Magnetic Bead Protein Coupling kit is a research use only kit for the purpose of enabling users to create their own assays using their own antibodies or proteins. The kit contains components required to build assays for two 96-well plates. It also lists other reagents and tools that are required to complete assay development.

Principle

The Applied BioCode System is a flexible multiplexing platform for detecting and analyzing targets using the Barcoded Magnetic Beads (BMB). A wide variety of assay types, such as nucleic acid hybridization assays, and immunoassays are performed in an aqueous, both quickly and efficiently.

The BioCode Analyzer and BMB technology offers multiplex capability for simultaneous detection up to 128 different analytes within a single sample.

With BMB technology, nucleotide/antibody reactions take place on the surface of BMB. For each sample, target specific capture nucleotide/antibody probes are covalently linked on to a specific set of BMB: (1) biotin labeled targets are captured by the BMB-bound oligo probes in a hybridization assay, (2) analyte/antigen captured by a specific capture probe coupled on BMB will in turn immobilize a biotin labeled detection antibody in a solid-phase immunoassay. Finally, the Streptavidin R-phycoerythrin conjugate is added to the samples for quantitative florescence detection.

The BioCode software completes the data collection and reports the results in a matter of minutes.

STORAGE and STABILITY

1. Kit is shipped at ambient temperature. Store remaining kit components at room temperature (RT, 15 to 30°C).
2. All components are guaranteed up to the expiration date found on the packaging label if handled properly.

SAFETY

1. Please refer to the product's Material Data Safety Sheet (MSDS) for safety information concerning this product.
2. Avoid reagent contact with skin. If contact is made, thoroughly wash the area with water.
3. Handle all specimens in accordance with Universal Precautions. (CDC, Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Bloodborne Pathogens in Health-Care Settings. MMWR 1988; 37:377-388. OSHA standards: 1910.1030 (d) (l) and 1910.1030 (d) (3).)
4. Waste must be classified and disposed of in accordance with all Federal, State, and Local environmental regulations.

NECESSARY REAGENTS, MATERIALS and EQUIPMENT

Kit Contents

Reagent Description	Quantity
P-Carboxyl Barcoded Magnetic Beads (BMB), 24-plex. The BMB's surface is functionalized for protein/antibody coupling.	24 tubes, ~10,000 beads/tube in 0.5 mL PBST

Part #	BMB #	Included (check mark)		Part #	BMB #	Included (check mark)
64-R0112-**a**	0 to 23			64-R0112-**d**	72 to 95	
64-R0112-**b**	24 to 47			64-R0112-**e**	96 to 119	
64-R0112-**c**	48 to 71					

Activation Buffer, 15% Ethanol in 100 mM MES, 0.05% ProClin, pH 6.0	1 vial, 20 mL
Coupling Buffer, 140 mM Guanidine-HCl, 100 mM MES, 0.05% ProClin 950, pH 5.0	1 vial, 20 mL
PBS, 1X, 0.05% ProClin 950, pH 7.4	1 vial, 20 mL

PBST, 0.05% Tween-20, in PBS w/ 0.05% ProClin 950, pH 7.4	1 bottle, 450 mL
TRIS Buffer, 50 mM TRIS-HCl, 0.05% ProClin 950, pH 7.4	1 vial, 20 mL
Tween/NaCl, 1 M NaCl, 0.01% Tween-20, 0.05% ProClin, pH 7.5	1 vial, 20 mL
Detection Buffer	1 bottle, 60 mL
96-well Pate/Cover	2 plates and 1 cover

Reagent Preparation (prepares fresh just before use, discard after use)

Reagent Description	**Protocol** (sufficient volume for 1 pate)
Blocking Buffer, 1% BSA, 1% NFDM in PBS	Dissolve 60 mg BSA and 60 mg NFDM in 6 mL PBS
Assay Buffer, 1% BSA in PBST	Dissolve 40 mg BSA in 40 mL PBST
EDC, 50 mg/mL	Add 200 μL Activation Buffer to EDC vial
Sulfo-NHS, 50 mg/mL	Dissolve 25 mg Sulfo-NHS in 0.5 mL Activation Buffer
SA-PE, 2 μg/mL	Dilute 10 μL of 1 mg/mL SA-PE in 5 mL Assay Buffer

Reagent Components and Supplies Not Provided

Description	Reference
Sulfo-NHS (N-hydroxysulfosuccinimide)	ProteoChem, catalog # C1102 or equivalent
EDC, 10 mg	ProteoChem, catalog # C1100 or equivalent
BSA (Bovine Serum Albumin), protease, DNAse free powder	Equitech Bio, catalog # BAH67 or equivalent
NFDM (non-fat dry milk)	Village Farm, Strum Foods, Inc or equivalent
SA-PE (Streptavidin R-phycoerythrin) with BSA, 1 mg/mL	MOSS Inc., catalog # SAPE-001 or equivalent
EZ-Link Sulfo-NHS Biotinylation Kit	Thermo Scientific, catalog # 21435
Zeba Spin Desalting Columns	Thermo Scientific, catalog # 89882

Required Equipment

Description	Reference
BMB Analyzer	Applied BioCode 1000A or 2000
Magnetic Stand, 12-positon	Promega, catalog # Z5342
Magnetic 96-well Plate Separator	Applied BioCode, Part # 01-M0001
Micro-centrifuge, bench-top	Galaxy Mini Star, VWR or equivalent
Vortex Mixer	VWR
Thermo Shaker, 96-well plate	Vortemp 56, Labnet or equivalent

Shaker, tube	BioShaker XP, Q Instruments or equivalent
Pipettor: P-20, P-200, P-1000, Multi-channel	

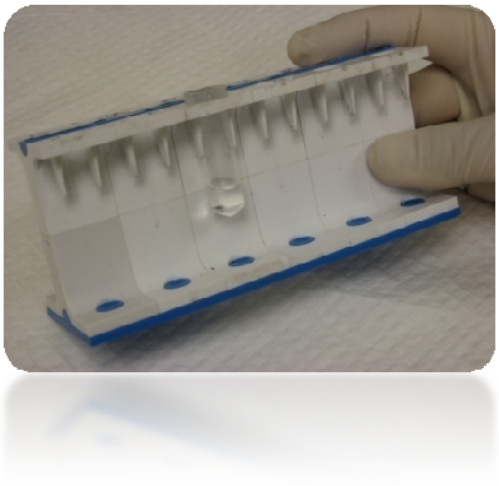
RECOMMENDED PROTOCOLS

Antibody Coupling

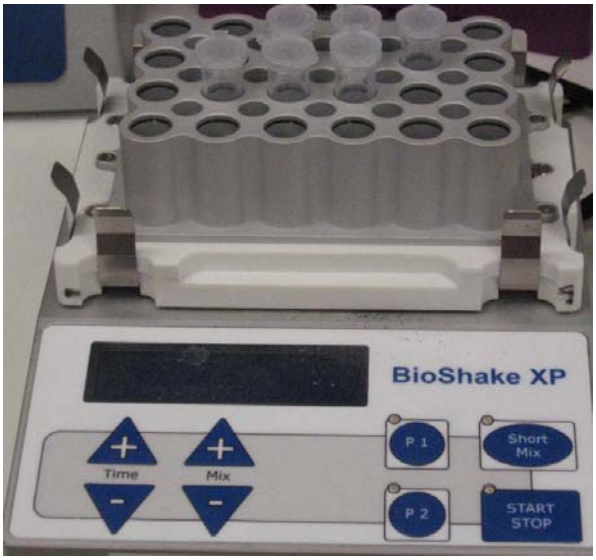
Technical Notes

- The process is for coupling antibodies or similar proteins to carboxylated BMB.
- The optimal binding capacity on the BMB may depend on the robustness of the antibody and the pH of Activation and Coupling Buffers. This kit includes the Activation Buffer (pH 6) and Coupling Buffer (pH 5) which is suitable for most antibodies.
- If the antibody is stored in buffers containing free amines, such as TRIS or Glycine, should be removed using a desalting column before coupling.
- For coupling, we recommend using 2 to 10 μg of antibody/protein per 10,000 beads.
- Prepare the EDC solution immediately before use.

1. Spin down and place the BMB tubes into the Magnetic Stand for 1 to 2 minutes. Note: Coupling up to 12 reactions can be performed simultaneously.
2. Remove the supernatant using a pipette.
3. Wash the BMB twice - add 200 μL of Activation Buffer into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
4. Repeat Step 3 once for a total of two washes.
5. Add 76 μL of Activation Buffer into the BMB tube.



6. Add 14 μL of 50 mg/mL Sulfo-NHS into the BMB tube, gently vortex the tube for 3 to 5 seconds.
7. Add 10 μL of 50 mg/mL EDC into the BMB tube, gently vortex the tube for 3 to 5 seconds; centrifuge the BMB tube for 8 to 10 seconds.
8. Place the BMB tube in a shaker for 20 to 30 minutes, mix at 1200 to 1500 rpm, at RT.



9. Remove the Activation Buffer from the BMB tube, follow Steps 1 and 2.
10. Wash the BMB twice – add 200 μL of Coupling Buffer into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
11. Repeat Step 10 once for a total of two washes.
12. Add 100 μL of Antibody (or Protein) diluted in Coupling Buffer into the BMB tube.
13. Place the BMB tube in a shaker for 2 hours, mix at 1200 to 1500 rpm, at RT.
14. Remove the supernatant, follow Steps 1 and 2.
15. Wash the BMB once – add 200 μL of Tween/NaCl into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
16. Add 200 μL of PBST into the BMB tube.
17. Place the BMB tube in a shaker for 13 to 15 minutes, mix at 1200 to 1500 rpm, at RT.
18. Remove the supernatant, follow Steps 1 and 2.
19. Wash the antibody/BMB tube twice – add 200 μL of TRIS Buffer into the BMB tube.
20. Place the BMB tube in a shaker for 30 minutes, mix at 1200 to 1500 rpm, at RT.
21. Remove the supernatant, follow Steps 1 and 2.
22. Repeat Steps 19 to 21 except shake the BMB tube for 10 minutes. For a total of two washes.

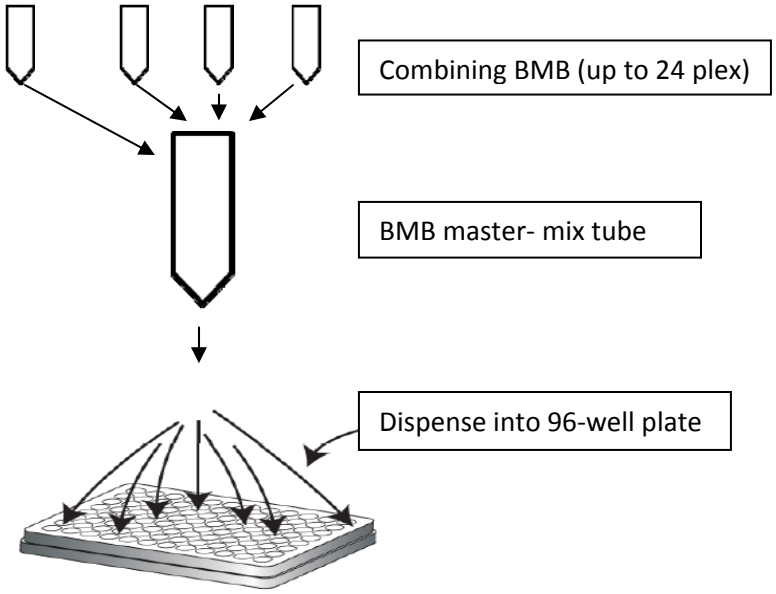
23. Wash the antibody/BMB tube once – add 200 μL Tween/NaCl into the BMB tube.
24. Place the BMB tube in a shaker for 3 to 5 minutes, mix at 1200 to 1500 rpm, at RT
25. Remove the supernatant, follow Steps 1 and 2.
26. Wash the antibody/BMB tube once – add 200 μL of Blocking Buffer, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
27. Wash the antibody/BMB tube once – add 200 μL of Blocking Buffer into the BMB tube.
28. Place the BMB tube in a shaker for 30-60 minutes, mix at 1200 to 1500 rpm, at RT.
29. Remove the supernatant, follow Steps 1 and 2.
30. Wash the antibody/BMB tube twice - add 200 μL of Assay Buffer into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
31. Repeat Step 30 once for a total of two washes.
32. Add 650 μL of Assay Buffer into the BMB tube.
33. Store BMB tube at 2 to 8°C until needed.

Biotinylation of Detection Antibodies

1. Recommended to use the Thermo Scientific Sulfo-LC Biotinylation kit.
2. Store the Biotin-Antibody in Assay Buffer at 2 to 8°C.
3. The Biotin-Antibody working concentration should be optimized.

Immunoassay Protocol

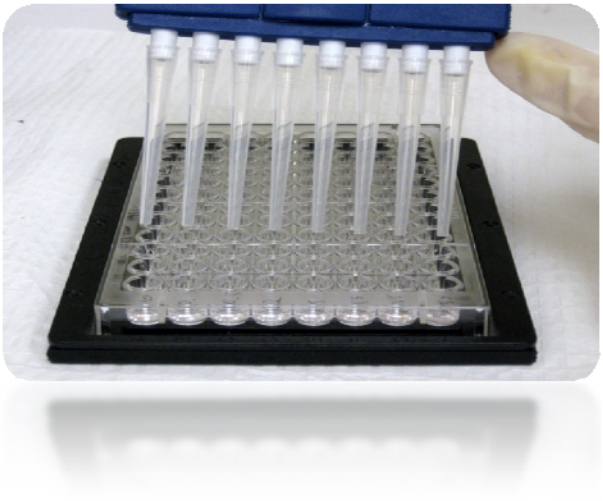
1. Recommend to use approximately 50 beads/plex per well.
2. Calculate the BMB volume needed for number of run(s) with 50 beads/plex per well.
3. Combine the BMB volume of each plex (analyte) into a master-mix tube.
4. As the example above, vortex each Stock Antibody/BMB tube until the beads are suspended, immediately transfer 0.31 mL into a 10-mL tube (Master-mix). Continues this transfer process until the desired plex numbers are in the Master-mix tube.
5. If necessary, add Assay Buffer to the Master-mix tube to a final volume approximately 7.7 mL.



Notes:

- The 7.7 mL final volume is for running one full plate.
- Calculate the make-up volume if it is less than a full plate. Example if only running 30 wells assay:
  Final Vol. = 75 μL x number of wells x 1.05
  = 75 μL x 30 x 1.05 = **2.4 mL**

6. Transfer 75 μL of Antibody/BMB from the Master-mix tube into corresponding wells.
7. Place the BMB plate on the Magnetic Plate Separator for 1 to 2 minutes.
8. Remove the supernatant using a multi-channel pipette.



9. Add 50 µL of Calibrators / Unknown Samples diluted in Assay Buffer into the corresponding wells.
10. Place the covered BMB plate in a shaker for 1.5 – 2 hours, mix at 900 to 1000 rpm, at RT.

11. Remove the supernatant from the BMB plate, follow Steps 7 and 8.
12. Wash the BMB twice – add 200 µL of PBST per well, follow Steps 5 and 6.
13. Add 50 µL of Biotin-Antibody diluted in Assay Buffer into each well.
14. Place the BMB plate in a shaker for 1 hour, mix at 900 to 1000 rpm, at RT.
15. Remove the supernatant from the BMB plate, follow Steps 5 and 6.
16. Wash the BMB twice as Step 10.
17. Add 50 µL of 5 µg/mL SA-PE into each well,
18. Place the covered BMB plate in a shaker for 30 minutes, mix at 900 to 1000 rpm, at RT.
19. Remove the supernatant from the BMB plate, follow Steps 7 and 8.
20. Wash the BMB 3 times as Step 10.
21. Add 200 µL of Detection Buffer into each well.
22. Place the covered BMB plate in a shaker for 2 minutes, mix at 700 rpm, at RT.
23. Measure the fluorescence intensity in the BioCode 1000A or 2000 Analyzer.

TECHNICAL SERVICE AND ORDERING INFORMATION

1. Customer Service: 562-801-0050
2. Ordering: Please call 562-801-0050 ext. 253 or email your orders to orders@ApBioCode.com
3. Bulk reagent order:

Description	Size	Part Number
Activation Buffer, pH 6.0	100 or 250 mL	44-A0501
Coupling Buffer, pH 5.0	100 or 250 mL	44-C0501
PBS, 1X, pH 7.4	450 or 950 mL	44-P0501
PBST, pH 7.4	450 or 950 mL	44-P0502
TRIS Buffer, pH 7.4	100 or 250 mL	44-T0502
Tween/NaCl, pH 7.5	100 or 250 mL	44-T0503
Detection Buffer	500 or 1000 mL	44-D0002
96-well Plate	10 plates/pack, 2 plate covers	01-P0002, 01-P0004
P-Carboxyl BMB	50,000 beads/tube	44-B0112

DISTRIBUTED BY:

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