Applied BioCode

24-Plex Carboxyl Barcoded Magnetic Beads Nucleic Acid Coupling Kit

Nucleic Acid Coupling and Hybridization Instructions

Part Number: 64-R0102-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 nucleic acid probes. 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 antibody 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.

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
Carboxyl Barcoded Magnetic Beads (BMB), 24-plex. The BMB's surface is functionalized for nucleic acid probe 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					

MES Buffer, 50 mM MES, 0.05% ProClin, pH 5.0	1 vial, 10 mL
MES-T Buffer, MES Buffer w/ 0.01% Tween-20, pH 5.0	1 vial, 20 mL
PBS, 1X, 0.05% ProClin 950, pH 7.4	1 vial, 20 mL
PBST, 0.1% Tween-20, in PBS, pH 7.4	1 bottle, 450 mL
TRIS Buffer, 50 mM, 0.05% ProClin 950, pH 7.4	1 vial, 20 mL
TMAC Buffer, 3M TMAC, 50 mM Tris-HCl, 0.1% Sarkosyl, 1% EDTA, 0.05% ProClin 950, pH 8.0	1 vial, 20 mL
Detection Buffer	1 bottle, 60 mL
96-well Pate/Cover	2 plates and 1 cover

Reagent Preparation

Reagent Description	**Protocol** (sufficient volume for 1 pate)
Blocking Buffer, 1% BSA, 1% NFDM in PBS	Dissolve 6 mg BSA and 6 mg NFDM in 6 mL PBS. Prepare fresh just before use, discard after use.
100 µM Amino-modified Oligo Probe	Dissolve in Nuclease-free Water. Store aliquots at -80°C.
Biotinylated Target Oligo Probe / Amplicon	Diluted in Nuclease-free Water or 10 mM TRIS Buffer (dilute the 50 mM TRIS). The optimal Target Oligo Probe concentration should be determined by the user. Store aliquots at -80°C.
EDC, 10 mg/mL	Add 1.0 mL MES Buffer to EDC vial. Prepare fresh just before use, discard after use.
SA-PE, 12 µg/mL	Dilute 1.2 µL of 1 mg/mL SA-PE in 97 µL TMAC Buffer, this volume is sufficient for 10 wells. Prepare fresh just before use, discard after use.

Reagent Components and Supplies Not Provided

Description	Reference
Nuclease-free Water, Ambion	Life Technologies, catalog # 9932 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
Zeba Spin Desalting Columns	Thermo Scientific, catalog # 89882 if required

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 or equivalent
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	
Plate Washer (optional)	BioTek Plate Washer Elx50 with magnetic separator

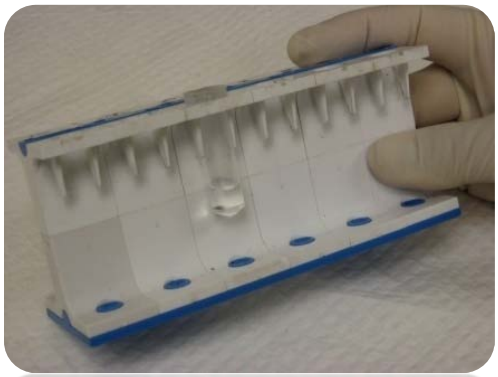
RECOMMENDED PROTOCOLS

Oligo Probe Coupling

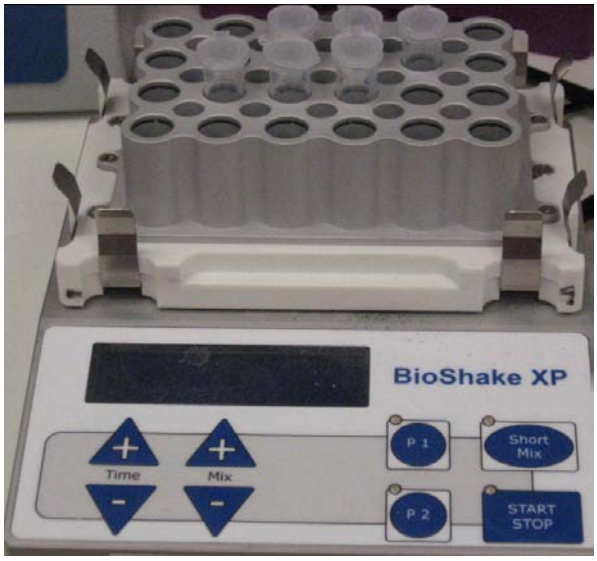
Technical Notes

- The process is for coupling Amino-modified Nucleic Acids to carboxylated BMB.
- The optimal binding capacity on the BMB may depend on the robustness of the Amino-modified Oligo Probes and the pH of MES Buffers. This kit includes the MES (Coupling Buffer), pH 5 which is suitable for most Oligo Probes. Researchers are advised to try an alternate Coupling Buffer and/or pH if necessary.
- If the Oligo Probe 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 100 pmol of Oligo Probe per 10,000 beads.
- Prepare the EDC solution immediately before use.

1. Quick spin, 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 MES-T 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 79 μL of MES-T Buffer into the BMB tube.



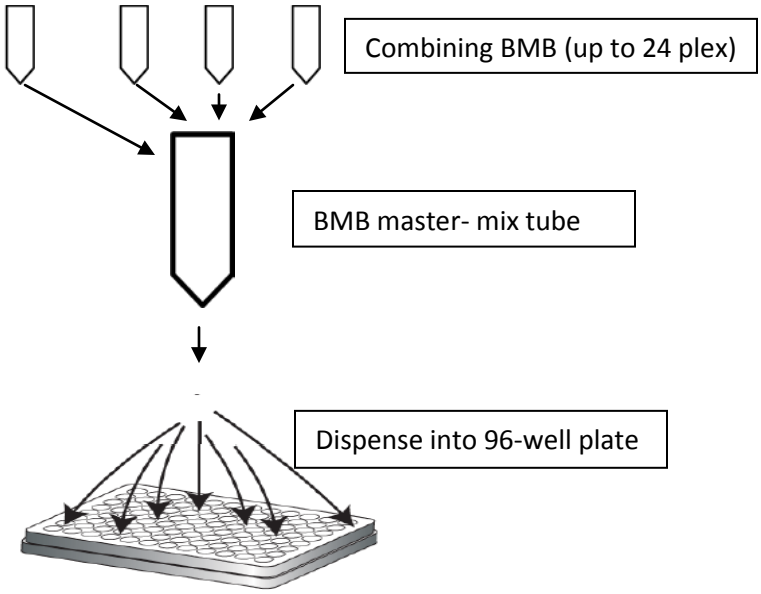
6. Add 1.0 μL of 100 μM Amino-modified Oligo Probe (100 pmol), gently vortex the tube for 3 to 5 seconds.
7. Add 20 μL of 10 mg/mL EDC into the BMB tube, gently vortex the tube for 3 to 5 seconds; centrifuge the BMB tube for 8 to 10 seconds.
8. Place the BMB tube in a shaker for 120 ± 10 minutes, mix at 1400 to 1600 rpm, at RT.



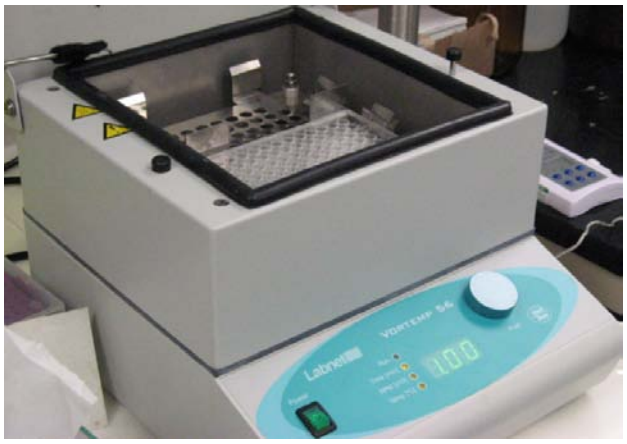
9. Remove the EDC/MES Buffer from the BMB tube, follow Steps 1 and 2.
10. Add 200 μL of TRIS Buffer into the BMB tube, place the tube in a shaker for 15 to 20 minutes, mix at 1400 to 1600 rpm, at RT.
11. Wash the BMB once – add 200 μL of Blocking Buffer into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
12. Block BMB – add 200 μL of Blocking Buffer into the BMB tube place the tube in a shaker for 60 ± 5 minutes, mix at 1400 to 1600 rpm, at RT.
13. Remove the Blocking Buffer from the BMB tube, follow Steps 1 and 2.
14. Wash the BMB twice – add 200 μL of PBST Buffer into the BMB tube, gently vortex the tube for 3 to 5 seconds. Repeat Steps 1 and 2.
15. Add 200 μL of PBS-T into the BMB tube.
16. Determine the BMB counts on the Applied BioCode 1000A or 2000 Analyzer.
    a. Transfer 1 to 2 μL of BMB per analyte into each well.
    b. Add 200 μL of Detection Buffer per well.
    c. Measure on the Analyzer.
17. Store the BMB at 2 to 8°C or proceed to hybridization steps.

Hybridization 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. Place the master-mix tube into the Magnetic Stand for 1 to 2 minutes.
5. Remove the supernatant using a pipette.
6. Add TMAC Buffer (TMAC volume = number of wells x 50 μL/ well) into the master-mix tube.



7. Continuously mix the master-mix tube on a vortex (the beads are suspended), use a pipette; transfer 45 to 48 μL BMB suspension into each well.
8. Add 2 to 5 μL of Biotinylated Target Oligo Probe / Amplicon into corresponding wells.
9. Place the covered BMB plate in a shaker for 15 to 20 minutes, mix at 675 to 725 rpm, at 52°C.
10. Prepare the 12 μg/mL SA-PE in TMAC Buffer.
11. Add 10 μL of 12 μg/mL SA-PE Working Solution per well.
12. Place the covered BMB plate in a shaker for 5 to 10 minutes, mix at 675 to 725 rpm, at 52°C.

13. Place the BMB plate on the Magnetic Plate Separator for 1 to 2 minutes.
    Optional – Wash the BMB on a BioTek Washer, 2 times with PBST.

14. Remove the supernatant using a multi-channel pipette.
15. Wash the BMB twice – add 200 μL of PBST per well, follow Steps 12 and 13.
16. Add 200 μL of Detection Buffer into each well.
17. Place the covered BMB plate in a shaker for 2 minutes, mix at 700 rpm, at RT.
18. 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
MES, 50 mM, pH 5.0	100 or 250 mL	44-M0501
MES-T, 50 mM, pH 5.0	100 or 250 mL	44-M0502
PBS, 1X, pH 7.4	450 or 950 mL	44-P0501
PBS-T, pH 7.4	450 or 950 mL	44-P0502
TRIS Buffer, 50 mM, pH 7.4	100 or 250 mL	44-T0502
Detection Buffer	500 or 1000 mL	44-D0002
TMAC Buffer, 3M, pH 8.0	100 or 250 mL	44-T0501
96-well Plate	10 plates/pack, 2 plate covers	01-P0002, 01-P0004
Carboxyl BMB	50,000 beads/tube	44-B0102

DISTRIBUTED BY:

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