Highly Scalable Barcoded Magnetic Beads for Multiplexed Assays
(2 → 32 → 128 → 1,024-plex)

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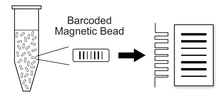
Digital Multiplexing System Offers Testing Solutions for Molecular Diagnostics, Biomarkers Validation and Companion Diagnostics with High Throughput, Accuracy, and Affordability

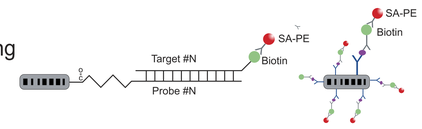
Introduction

Barcoded Magnetic Beads (BMBs) utilize digital technology instead of conventional analog methodology to offer unmatched decoding accuracy, precise fluorescence detection, and a virtually unlimited number of barcodes for use in multiplex tests. The marriage of BMB technology and biotechnology results in an extremely flexible platform suited for a wide variety of biological applications, such as clinical diagnostics, gene mutation analysis, drug resistance genotyping, and pharmaceutical drug discovery. The polymer-based BMB (60-100 µm x 25 µm x 6 µm) is manufactured using a well-established and highly reproducible semiconductor lithographic process. All BMBs are created equal, with few bead-to-bead and lot-to-lot variations. BMBs are permanently encoded with paramagnetic material, which not only provides the digital pattern for accurate decoding, but also enables easy washing, separation, and automation. The digital pattern is highly scalable, allowing 2^N (N= number of digits) unique codes. BMBs with N = 5, 7, and 10 have been mass produced. These beads show a "high-contrast transmitted barcode" pattern when illuminated with a light source.

Applications

- Infectious disease testing
- Biomarker validation & pharmacogenomics
- Cancer diagnostics
- Companion diagnostics
- Gene expression & genetic testing





Advantages

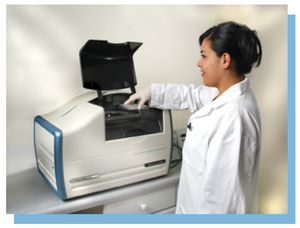
- **Flexible, customizable probe addition** - BMB's flexibility enables new probes to be affixed to beads quickly and easily.
- **Robust system** - Easy to use because no complicated laser or flow cytometer is needed. Absolutely no sample beads carry-over.
- **Excellent decoding accuracy** - High contrast digital barcode enables accurate decoding.
- **Rapid reaction** - Provides solution-phase kinetics and 15-minute hybridizations.

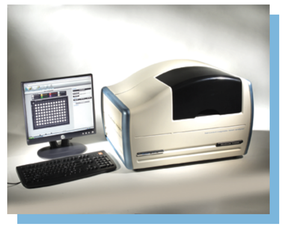
Barcoded Magnetic Beads

Three types of beads are available: (1) a standard BMB for physical adsorption, (2) a carboxyl BMB for covalent attachment, and (3) a streptavidin BMB for SA-biotin coupling. Carboxyl beads permit probes and specific primers to bind the bead surface covalently via NH_2-modified 5' termini. All bead types enable attachment of probes that are proteins, peptides, nucleic acids, and other ligands in a highly multiplexed format with high stability and low nonspecific binding. This simple and flexible immobilization chemistry enables rapid assay development for a variety of applications.

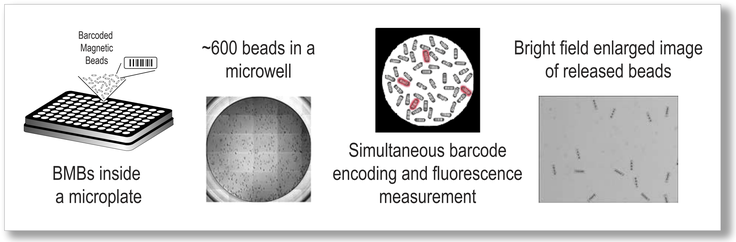
The BioCode-1000 Analyzer (2 to 128-plex)

The BioCode-1000 Analyzer is a rapid, simple, and robust BMB imaging system for a 96-well microplate format. The system is used to detect 2 to 128-plex targets in individual microwells. After hybridization,the binding reaction, beads settle to the bottom of each well, allowing for barcode identification and fluorescent signal detection. Multiple analyte tests can be performed in each well. The BioCode-1000 utilizes a CCD camera that detects both bright field barcode signal and reaction fluorescence signal. Positive or negative biochemical reactions are reported based on fluorescence intensityThe BioCode-1000 rapidly displays the barcode and fluorescence (phycoerythrin (PE)) intensity for each BMB, which enables clear reporting of reaction results for each probe. Other fluorophores can also be accommodated by switching optical filter sets. The system's image analyzer can scan barcodes and detect fluorescent signal for every bead in a microwell in 40 seconds, processing 96 samples in one hour.





BioCode-1000 Analyzer is rebust and simple to use for 2 ~ 128-plex assays.



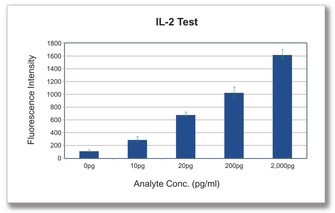
The BioCode-HP Analyzer (high plex: 1,024-plex) (in development)

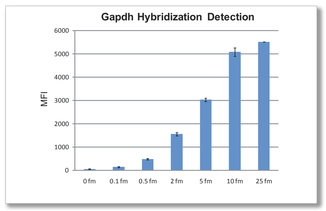
The BioCode-HP system enables researchers to evaluate a larger number of targets per sample (up to 1,024-plex). In this system, beads are re-distributed on a larger surface image plate after hybridization, where a high speed detector reads barcodes and measures fluorescent signal. More than 50,000 beads can be read in less than one minute. Proprietary software is used to read barcodes and detect fluorescent signal for each bead on the image plate.

Results

This system is extremely sensitive (0.5 fm/ml GAPDH (DNA) and 1.0 pg/ml IL-2 (protein) with PE label) and has been used for a variety of panel tests. Shown below are results from a 10-plex cytokine immunoassay panel; 18-plex for identification of Gram-positive staphylococci bacteria and drug-resistance genes; and 8-plex thrombo SNP targets.

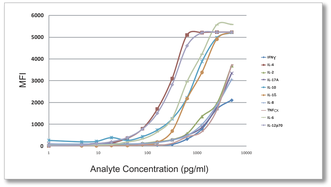
Sensitivity Studies of BMB-based Bioassay





Left: IL-2 and and **Right**: Oligonucleotide GAPDH (50-mer). This system achieves excellent sensitivities of 0.5 fm/ml GAPDH (DNA) and 1.0 pg/ml IL-2 (protein).

Multiplex Detection Range of 10-plex Cytokine



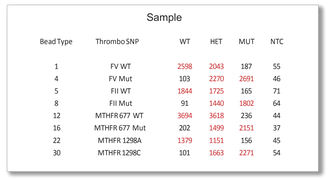
Serial dilutions of multiplex protein standard were analyzed using BMB technology. Signal intensities of each cytokine-bead type are plotted for all cytokine concentrations measured (5 pg/ml to 5000pg/ml).

Specificity - 10-plex Cytokine Immunoassay

10-Plex		Analytes									
Barcode	Target	IFNγ	IL-4	IL-2	IL-17A	IL-10	IL-1ß	IL-8	TNFα	IL-6	IL-12p70	CT
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
0	IFNγ	2108	93	71	116	185	162	116	93	116	176	116
6	IL-4	63	5583	95	63	63	31	95	95	130	63	139
8	IL-2	116	116	3806	148	139	139	116	139	139	130	116
10	IL-17A	116	116	116	3510	139	207	162	162	139	132	116
11	IL-10	95	95	63	95	5583	159	125	95	95	123	125
12	IL-1ß	93	230	162	93	162	5583	162	139	162	133	139
13	IL-8	139	116	116	116	162	185	5014	162	139	153	116
26	TNFα	116	162	116	116	207	207	185	5583	162	136	139
27	IL-6	139	185	93	185	185	176	139	162	5583	152	139
29	IL-12p70	139	139	93	116	207	121	139	139	116	5583	93

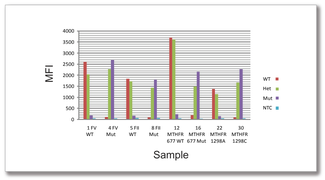
This immunoassay tests the specificity of the 10-plex cytokine immunoassay using 10 different anti-cytokine capture antibodies conjugated with 10 different barcoded BMBs. The BMBs were pooled to make a working mix and mixed with each analyte in the well indicated. After adding a mixture of biotin-labeled detection antibodies and SA-PE, the BMBs were washed and scanned by the Biocode-1000. The resulting data clearly show detection of each analyte by corresponding capture/detection antibodies with minimum background signals.

Thrombo SNP Genotyping



Sample

Bead Type	Thrombo SNP	WT	HET	MUT	NTC
1	FV WT	2598	2043	187	55
4	FV Mut	103	2270	2691	46
5	FII WT	1844	1725	165	71
8	FII Mut	91	1440	1802	64
12	MTHFR 677 WT	3694	3618	236	44
16	MTHFR 677 Mut	202	1499	2151	37
22	MTHFR 1298A	1379	1151	156	45
30	MTHFR 1298C	101	1663	2271	54



The EraGen MultiCode®-PLx assay has been used in conjunction with BMB technology for the detection of thrombo SNP targets. The eight targets representing wild type and corresponding SNP mutants of three genes (FV, FII, MTHFR) were amplified and labeled using a multiplex primer pool. The resulting extension products were hybridized to BMBs, then analyzed on the BioCode-1000 Analyzer.

Conclusion

Barcoded Magnetic Beads (BMBs) utilize digital technology instead of conventional analog methodology to offer unmatched decoding accuracy and fluorescence detection for precision multiplex tests. The marriage of BMB technology and biotechnology results in a very flexible platform for routine clinical immunoassays, molecular diagnostics, companion diagnostics, biomarker validation, and pharmacogenomics. The Biocode-1000 Analyzer can scan a 96-well microplate, decode barcodes, measure label fluorescence, and display results in only 40 seconds per microwell. The Biocode-1000 Analyzer simplifies multiplex assays while offering high throughput, high accuracy, and affordability.

This poster was presented at the 42nd Annual AACC Oak Ridge Conference from April 22 ~ April 23, 2010 at San Jose, CA.