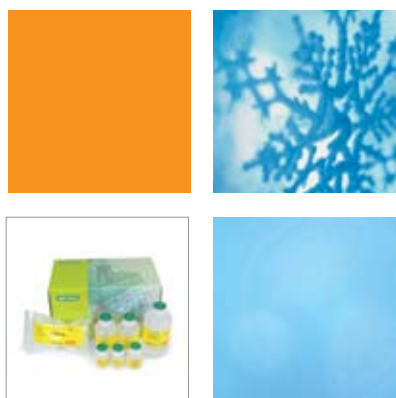




# Bio-Plex® System Bead Coupling



Unlimited Design



# Infinite Possibilities for Multiplex Assay

## Design Your Own Assays

Bead coupling is an integral component of developing bead-based multiplex assays. Bio-Rad offers a selection of 100 Bio-Plex COOH beads for use in assay development. The coupling procedure involves a rapid two-step carbodiimide reaction between primary amine groups on the protein and carboxyl functional groups on the bead surface. With your choice of Bio-Plex COOH beads, you can:

- Covalently couple 6–150 kD proteins or oligonucleotides in <5 hours
- Couple antibodies, antigens, receptors, ligands, enzymes, or nucleic acids
- Choose from a variety of bead regions
- Couple protein for up to 20 microplates with each milliliter

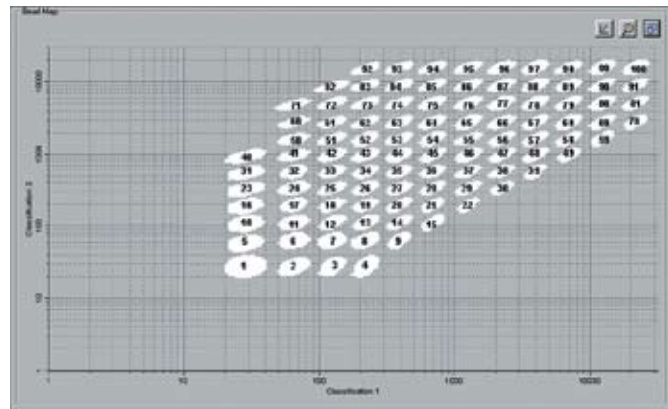
The coupled beads can then be used in multiplex protein-protein binding studies or in the development of unique multiplex assays that can be analyzed with the Bio-Plex suspension array system.

## The Complete Bead Selection

Bio-Plex COOH beads are carboxy-coated beads internally labeled with two fluorescent dyes (xMAP technology). The complete selection of 100 spectrally distinct beads is available from Bio-Rad in 1 ml tubes and 16 ml bottles.

Each milliliter of Bio-Plex COOH beads ( $1.25 \times 10^7$  beads/ml) is sufficient for ten coupling reactions using 5,000 beads per well. You can covalently couple your protein or oligonucleotide to the surface of each dyed bead, with enough beads for up to 2,000 data points, or twenty 96-well microplates.

For more information about Bio-Plex bead coupling products, contact your local Bio-Rad sales representative or visit us on the Web at [www.bio-rad.com/bio-plex/](http://www.bio-rad.com/bio-plex/)



Map of 100 bead regions.



# Development

## For One-of-a-Kind Assays

If you have access to a Luminex instrument, or are using ELISA or western blots but would like to develop your own multiplex assays, the Bio-Plex COOH beads can help you attain your goals. These beads can be used to develop unique bead-based multiplex assays specific to your research needs. We recommend Bio-Plex COOH beads for researchers who:

- Are testing proprietary or unique targets or samples
- Require a broader assay selection than is commercially available
- Require flexibility to change targets often
- Require assay customization
- Have assay development resources

### Complement Your Bio-Plex Assay

Bio-Plex COOH beads can be used to develop cytokine or phosphoprotein assays not currently available from Bio-Rad. Bio-Rad also offers a selection of related reagent and sample preparation kits to complement your Bio-Plex assays. The Bio-Plex cytokine reagent kit and the Bio-Plex serum diluent kits can be used for cytokine assay development. Similarly, the Bio-Plex phosphoprotein detection reagent kit and the Bio-Plex cell lysis kit can be used for phosphoprotein assay development. These assays can then be mixed with existing Bio-Plex cytokine or phosphoprotein assays to create a customized multiplex assay.

### An Open Platform

The Bio-Plex COOH beads can be used to develop assays that complement any Luminex-based assay on the market. In addition, any Luminex-based assay can be read on the Bio-Plex suspension array system; conversely, Bio-Plex assays can be used with any Luminex instrument.

## Three Steps to Successful Bead Coupling

### 1. Protein Preparation

Water-soluble proteins ranging in size from 6 to 150 kD must be suspended in PBS, pH 7.4, and must be free of sodium azide, BSA, glycine, Tris, or amine-containing additives.

### 2. The Coupling Reaction

Carboxyl groups on the surface of the polystyrene beads are first activated with the carbodiimide derivative EDC\* to form an intermediate that is stabilized with S-NHS. The intermediate then reacts with a protein's primary amine to form an amide bond. The protein-coupled beads are stable for 1 year when stored at 4°C and protected from light.

### 3. Coupling Validation

The protein-coupled beads are counted, and the efficiency of the coupling reaction is assessed with the Bio-Plex suspension array system, or any Luminex instrument.

#### Coupling Protocol Details for Bio-Plex COOH Beads

Bead surface chemistry	Carboxyl functional group
Capture molecule	Amine group of protein/peptides
Binding chemistry	Covalent binding using EDC/S-NHS
Immobilization	Random
Coupling optimization	Required
Activation step	EDC/S-NHS (20 min)
Incubation	2 hr or overnight
Wash step	1 wash step
Additional steps	Block (30 min); count beads
Coupled bead stability	1 year
Bead volume (per coupling)	100 µl
Number of beads (per coupling)	1.25 x 10 <sup>6</sup>
Protein required (per coupling)	5–12 µg; depends on protein used
Data points (per coupling)	125–200
Coupling yield	50–80%
Beads/well	5,000
Data points (per µg protein)	10–40; depends on protein used

\* EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) and S-NHS (N-hydroxysulfosuccinimide)



# Ordering Information

## How to Order

Each milliliter of Bio-Plex COOH beads contains enough beads for ten coupling reactions. This volume is sufficient to produce enough protein-coupled beads for up to twenty 96-well microplates using 5,000 beads per well.



Catalog #	Description
<b>Beads</b>	
(See table)	<b>Bio-Plex COOH Beads</b> , 1.25 x 10 <sup>7</sup> beads/ml, 1 ml
(See table)	<b>Bio-Plex COOH Beads</b> , 1.25 x 10 <sup>7</sup> beads/ml, 16 ml
<b>Reagent Kits</b>	
171-304000	<b>Bio-Plex Cytokine Reagent Kit</b> , 1 x 96-well
171-304001	<b>Bio-Plex Cytokine Reagent Kit</b> , 10 x 96-well
171-304004	<b>Bio-Plex Phosphoprotein Detection Reagent Kit</b> , 1 x 96-well
171-304005	<b>Bio-Plex Phosphoprotein Detection Reagent Kit</b> , 10 x 96-well
<b>Sample Preparation Kits</b>	
171-304011	<b>Bio-Plex Cell Lysis Kit</b> , 1 x 96-well
171-304012	<b>Bio-Plex Cell Lysis Kit</b> , 10 x 96-well
171-305000	<b>Bio-Plex Human Serum Diluent Kit</b> , 1 x 96-well
171-305001	<b>Bio-Plex Human Serum Diluent Kit</b> , 10 x 96-well
171-305004	<b>Bio-Plex Mouse Serum Diluent Kit</b> , 1 x 96-well
171-305005	<b>Bio-Plex Mouse Serum Diluent Kit</b> , 10 x 96-well
171-305008	<b>Bio-Plex Rat Serum Diluent Kit</b> , 1 x 96-well

## COOH Beads

	Catalog #			Catalog #	
	1 ml	16 ml		1 ml	16 ml
1	171-506001	171-606001	51	171-506051	171-606051
2	171-506002	171-606002	52	171-506052	171-606052
3	171-506003	171-606003	53	171-506053	171-606053
4	171-506004	171-606004	54	171-506054	171-606054
5	171-506005	171-606005	55	171-506055	171-606055
6	171-506006	171-606006	56	171-506056	171-606056
7	171-506007	171-606007	57	171-506057	171-606057
8	171-506008	171-606008	58	171-506058	171-606058
9	171-506009	171-606009	59	171-506059	171-606059
10	171-506010	171-606010	60	171-506060	171-606060
11	171-506011	171-606011	61	171-506061	171-606061
12	171-506012	171-606012	62	171-506062	171-606062
13	171-506013	171-606013	63	171-506063	171-606063
14	171-506014	171-606014	64	171-506064	171-606064
15	171-506015	171-606015	65	171-506065	171-606065
16	171-506016	171-606016	66	171-506066	171-606066
17	171-506017	171-606017	67	171-506067	171-606067
18	171-506018	171-606018	68	171-506068	171-606068
19	171-506019	171-606019	69	171-506069	171-606069
20	171-506020	171-606020	70	171-506070	171-606070
21	171-506021	171-606021	71	171-506071	171-606071
22	171-506022	171-606022	72	171-506072	171-606072
23	171-506023	171-606023	73	171-506073	171-606073
24	171-506024	171-606024	74	171-506074	171-606074
25	171-506025	171-606025	75	171-506075	171-606075
26	171-506026	171-606026	76	171-506076	171-606076
27	171-506027	171-606027	77	171-506077	171-606077
28	171-506028	171-606028	78	171-506078	171-606078
29	171-506029	171-606029	79	171-506079	171-606079
30	171-506030	171-606030	80	171-506080	171-606080
31	171-506031	171-606031	81	171-506081	171-606081
32	171-506032	171-606032	82	171-506082	171-606082
33	171-506033	171-606033	83	171-506083	171-606083
34	171-506034	171-606034	84	171-506084	171-606084
35	171-506035	171-606035	85	171-506085	171-606085
36	171-506036	171-606036	86	171-506086	171-606086
37	171-506037	171-606037	87	171-506087	171-606087
38	171-506038	171-606038	88	171-506088	171-606088
39	171-506039	171-606039	89	171-506089	171-606089
40	171-506040	171-606040	90	171-506090	171-606090
41	171-506041	171-606041	91	171-506091	171-606091
42	171-506042	171-606042	92	171-506092	171-606092
43	171-506043	171-606043	93	171-506093	171-606093
44	171-506044	171-606044	94	171-506094	171-606094
45	171-506045	171-606045	95	171-506095	171-606095
46	171-506046	171-606046	96	171-506096	171-606096
47	171-506047	171-606047	97	171-506097	171-606097
48	171-506048	171-606048	98	171-506098	171-606098
49	171-506049	171-606049	99	171-506099	171-606099
50	171-506050	171-606050	100	171-506000	171-606000

Bead packaged in 1 ml tubes are currently available off the shelf and will ship the next day (highlighted in orange). Orders for beads in 16 ml bottles will ship within 4-6 weeks (minimum volume requirements apply).

xMAP is a trademark of Luminex Corporation. The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.



**Bio-Rad  
Laboratories, Inc.**

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