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Carboxyl Terminated Magnetic Iron oxide Nanocrystals with Protein G (Catalog # IPG)

Product Specifications

Catalog # IPG: contains a proprietary Protein G (PG) conjugated carboxyl terminated magnetic iron oxide nanocrystals (IO) at 1:20 molar ratio of the IO to PG. The IPG formulation is in borate buffer at pH 7.4. IPG is available in 10, 15, 20, 30, and 50 nm diameter IO.

Product Introduction:

Ocean NanoTech's carboxyl terminated magnetic iron oxide nanocrystals (IO) of various diameters (10 nm-50 nm) are now available as protein G (PG) conjugated products (IPG). The protein G used for conjugation either a 32 kda recombinant PG or a IPG has been successfully used for the capture of anti-streptavidin IgG that had a HRP label forming IO-PG-Ab~HRP). The IO-PG-Ab~HRP was demonstrated to have activity over 3,3',5,5' tetramethylbenzidene (TMB) producing a blue product that turns yellow upon addition of an acid. The blue product is detected at 370 or 655 nm while the yellow product is detected at 450 nm. The amount of antibody captured by the IO-PG conjugate is directly proportional to the HRP activity after thorough washing to eliminate non-specifically adsorbed Ab~HRP on the nanocrystals. The IO-PG at 1:20 molar ratio captures a minimum of 10 antibodies per IO.

Table 1. Catalog of IPG products

Catalog #	PG MW	IO size (nm)	Volume
IPG-020-001	33-33kda	20	1 mL
IPG-020-005	33-33kda	20	5 mL
IPG-030-001	33-33kda	30	1 mL
IPG-030-005	33-33kda	30	5mL

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IPG-050-001	**33-33kda**	**50**	**1 mL**
IPG-050-001	**33-33kda**	**50**	**1 mL**

Sample Protocol: Application of the IPG for Antibody Capture

IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Materials

- 1mL of 1 mg/mL Magnetic Iron Oxide Nanocrystals Conjugated to Protein-G **(Catalog # IPG)**
- Wash/Storage buffer, 10 mL **(Catalog # WB)**
- Pipettes for delivering 500 uL volumes
- Vortex mixer capable of securing 1.5 mL tubes for incubations
- 4^{o}C Refrigerator
- SuperMag Magnetic Separation Device (**Catalog # SuperMag Separator™**) for IO less than 30 nm and the SuperMag Multi Tube Separation device (**Catalog # SuperMag MultiTube Separator™**) for 30 nm or bigger IO.
- 4 mL capacity plastic cuvettes for magnetic washes if using the SuperMag Separator™ separation device or 1.5 mL microcentrifuge tubes if using the SuperMag Multi Tube Separation device

Reagents Preparation:

NOTES:

1) Allow all reagents to come to room temperature before starting.

2) It is best to test your system with an enzyme-labeled antibody before using a non-labeled antibody in order to establish the IO-PG:Ab ratio that will work best for your system and to avoid aggregation.

1X Wash/Storage Buffer:

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Dilute 1mL of the 10X wash/storage buffer with 9 mL deionized water to give 10 mL of a 1X wash/storage buffer working solution. This will be sufficient for 1 x 1 mg magnetic iron oxide nanocrystals conjugation.

Antibody Solution:

Dissolve the antibody at 1 mg/mL in 1X wash/storage buffer which works best for the IO-PG that is shipped in the same buffer.

Procedure for Antibody capture with IPG:

1. Transfer at least 1 mg of IO-PG conjugate into a 1.5 mL microcentrifuge tube. Check Table 1 for number of moles present.
2. Magnetize to remove the supernatant solution.
3. Add the antibody solution to make a molar ratio of PG to Ab between 1:20 to 1:40 .
4. Incubate at 4^{o}C overnight.
5. Separate using a magnet.
6. Wash with the wash storage buffer three times making sure to collect all the supernatant solution after each wash.
7. Reconstitute the IO-PG-Ab~HRP with wash/storage buffer.

Establish the concentration of Antibody on the IO-PG surface using the HRP (or other enzymes) activity:

1. Transfer 98 uL of TMB into a 96-well plate.
2. Add 2 uL of the IO-PG-Ab~HRP and mix well.
3. Incubate at RT for 30 min (or less if the color appears sooner which means that the IO-PG-Ab~HRP is concentrated).
4. Add 10 uL of 0.3 N HCl and mix well.
5. Record the absorbance at 450 nm.
6. Establish the HRP concentration using a calibration standard.
7. Calculate the amount of HRP which is directly proportional to the concentration of Ab on the surface of the IO-PG.

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HRP calibration Standard:

1. Transfer 99 uL of TMB into a 96-well plate.
2. Add the various volumes of 50 ug/mL Ab~HRP as shown on the Table 2 and mix well.
3. Incubate at RT for 30 min (or less if the color appears sooner which means that the Ab~HRP is too concentrated).
4. Add 10 uL of 0.3 N HCl and mix well.
5. Record the absorbance at 450 nm.
6. Establish the calibration standard by plotting the Absorbance against the HRP concentration.
7. Calculate the concentration of Ab on the IO-PG-Ab~HRP using the slope of the curve.

Table 1. Molar concentration of Fe in the different sizes of IO Nanocrystals

Crystal Size (nm)	Nanocrystal Molar Concentration (nmol) of 1 mg Fe	Nanocrystal Molar Concentration (nmol) for 10 mg Fe	Nanocrystal Molar Concentration (nmol) for50 mg
5	7.26	72.6	363.0
10	0.91	9.1	45.5
15	0.27	2.7	13.5
20	0.11	1.1	5.5
25	0.058	0.58	2.9
30	0.034	0.34	1.7
35	0.021	0.21	1.05
40	0.014	0.14	0.7
45	0.010	0.1	0.5
50	0.0073	0.073	0.365

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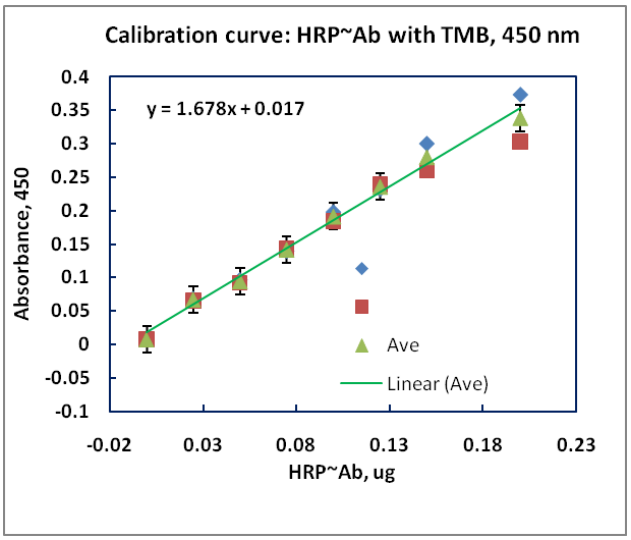
Table 2. Example of parameters and results of enzyme activity calibration standard for IO-PG antibody capture					
Volume of 50 ug/mL Ab~HRP	ug Ab~HRP	A 450 trial 1	A450 trial 2	Ave	Stdev
0.0	0.000	0.008	0.008	0.008	0.000
0.5	0.025	0.069	0.066	0.068	0.002
1.0	0.050	0.097	0.093	0.095	0.003
1.5	0.075	0.140	0.144	0.142	0.003
2.0	0.100	0.198	0.185	0.192	0.009
2.5	0.125	0.232	0.240	0.236	0.006
3.0	0.150	0.300	0.260	0.280	0.028
4.0	0.200	0.373	0.304	0.339	0.049



Related Ocean NanoTech Products

Visit our web site for additional information related to this product including the following items:

QSH, QSA, SHP, SHA, Blocking buffers (Catalog # BBB and BBG)

MNP KIT CATALOGUE #	MAGNETIC SEPARATION DEVICE
ICK-10-005	SuperMag Separator™
ICK-15-005	SuperMag Multitube Separator™
ICK-20-005	
ICK-25-005	
ICK-30-005	
ICK-40-005	
ICK-50-005	

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Current versions of product instructions are available at *www.oceannanotech.com*. For a faxed copy, call 479-431-4130 or 479-751-5500.