Protein A/G Terbium Fluorescent Magnetic Beads

BcMag™ Protein A/G Terbium Fluorescent Magnetic Beads (Fig.1) are Time-Resolved fluorescent (TRF) magnetic microspheres covalently coupled with ProteinA/G on the surface. The beads are manufactured using nanometer-scale superparamagnetic iron oxide and terbium metal as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide and Terbium metal. The microspheres combine the benefits of a novel avidin biotin-binding method, time-resolved fluorescent dyes, and magnetic characteristics to perform very sensitive assays.

Fig.1 BcMag™ Terbium Fluorescent Magnetic Beads

Protein A/G is a genetically engineered recombinant fusion protein that includes four Fc binding domains from Protein A and two from Protein G and has a final mass of 50.4 kDa. Protein A and Protein G are antibody-binding proteins derived from Staphylococcus aureus and Streptococcus sp., respectively. These proteins have different immunoglobulin-binding capacities and specificity. Protein A/G is a more potent protein exhibiting less pH-dependent binding and has binding profiles of both the proteins compared to Protein A or Protein G alone. Protein A/G strongly binds to human total IgG, IgG1, IgG2, IgG3, and IgG4, which is ideal for binding polyclonal or monoclonal IgG antibodies, whose subclasses are unsure yet. In the case of mouse IgG, Protein A/G can bind to all classes, including total IgG, IgG1, IgG2a, IgG2b, and IgG3 - except IgA, IgM, and serum albumin, allowing Protein A/G to efficiently purify and detect mouse monoclonal IgG antibodies, without cross-binding to IgA, IgM and serum albumin. Also, Protein A/G binds more powerfully and efficiently to mouse monoclonal antibodies than to Protein A or Protein G alone. The antibody binding properties are summarized in table 1.

Species	Antibody	Binding (Protein A/G)	Species	Antibody	Binding (Protein A/G))
Mouse	IgG 1	+++	**Sheep**	IgG1	++++
	IgG 3	++++		IgG2	++++
	IgG 2a	++++		Total IgG	++++
	IgG 2b	++++	**Horse**	IgG(ab)	++
	IgM	-		IgG(c)	++
	Total IgG	++++		IgG(T)	++++
Human	IgG1	++++		Total IgG	++++
	IgG2	++++	**Goat**	IgG1	++++
	IgG3	++++		IgG2	++++
	IgG4	++++		Total IgG	++++
	IgA	++	**Cow**	IgG1	++++
	IgD	-		IgG2	++++
	IgM	++		Total IgG	++++
	Fab	++	**Rabbit**	Total IgG	++++
	scFv	++	**Guinea Pig**	Total IgG	++++
	Total IgG	++++	**Pig**	Total IgG	++++
Rat	IgG 1	+++	**Cat**	Total IgG	++++
	IgG 2a	++++	**Dog**	Total IgG	++++
	IgG 2b	++	**++++ (Strong Binding); +++ (Medium Binding); ++ (Weak Binding); - (No Binding); N/A (No Information)**		
	IgG 2c	++++			
	Total IgG	+++			

Table 1. Protein A/G binding properties

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Although conventional fluorophores have been widely used over the past decades, they still suffer from either one or several limitations in terms of applicability and efficiency: 1. Narrow excitation bands cause higher background signals. 2. Smaller Stokes shift often produces self-quenching. 3. Fluorescence is sensitive to environmental factors such as metallic ion concentration, pH, temperature, and solvent polarity. 4. Fluorescence intensity is not high enough for detecting a single biomolecular. 5. Fluorescence intermittency (blinking) affects some processes of molecule detection. 6. Easily aggregated because of hydrophobicity.

Fluorescent dye properties

Fluorophore	Fluorescent color	Excitation (nm)	Emission (nm)	Fluorescence lifetime (ζ) (μsec)	Stokes shifts (nm)	Selection of Emission Filter
Terbium (Tb^{3+})	Green	320	545	1050	220	545/40

Specification	
Bead Size	2.5μm diameter; 5μm diameter
Number of Beads	~10 x 10^7 beads/mg (2.5μm), ~5 x 10^7 beads /mg (5μm)
Magnetization	~40-45 EMU/g
Type of Magnetization	Superparamagnetic
Concentration	10 mg/ml (10mM Tris, 0.15 M NaCl,0.1% BSA,1 mM EDTA, pH7.4)
Binding Capacity	~ 1 mg IgG/ml of Beads
Storage	Ship at room temperature. Store at 4°C. Do not freeze

BcMag™ TR-FRET (Time-Resolved FRET) Assay

BcMag™ TR-FRET Assay, in contrast to typical FRET (Förster Resonance Energy Transfer) assays, uses time-resolved fluorescent magnetic beads (BcMag™ TR-Magnetic Beads) as the donor fluorophore. The donor and acceptor can be two proteins, two DNA strands, an antigen and an antibody, or a ligand and its receptor. After a reasonable time delay (usually 50 to 100 s), a signal is generated by fluorescence resonance energy transfer between a donor and an acceptor molecule when they are close and monitored in a time-resolved way. In BcMag™ TR-FRET Assay, a trace amount of analytes can be easily enriched from the complex by TR-Magnetic Beads, resulting in higher sensitivity. This assay practically eliminates all fluorescence backgrounds caused by the sample and plastic microplate, as well as by direct acceptor excitation. As a result, the signal-to-noise ratios of the BcMag™ TR-FRET Assay are very high, and the background is quite low. Furthermore, the assay does not need washing steps. BcMag™ TR-FRET Assay offers substantial advantages to bioassays in high throughput screening, such as assay flexibility, dependability, increased assay sensitivity, higher throughput, and fewer false positive/false negative results.

Terbium cryptate fluorophore is an efficient fluorescent label due to its distinct specific properties. It is excited at 320nm and emits green fluorescence at 545nm, with a long fluorescence lifetime (1050 μsec) and large stokes shifts (220 nm). By taking advantage of these properties, time-resolved fluorescence measurement can dramatically reduce the fluorescence background from the sample and increase the signal-to-noise ratio to offer detectability better than one order of magnitude than conventional fluorescent dyes. BcMag™ Protein A/G Terbium Fluorescent Magnetic Beads are excellent donors used in TR-FRET assays.

Workflow of TR-FRET Magnetic Beads Assay (Fig.2)

1. Mix the antibody conjugated donor beads with the cell lysates and incubate them with continuous rotation for a sufficient time. The beads remain suspended in the sample solution during mixing, allowing the target analytes to bind to the donor beads. 2. After incubation, the beads are collected and separated from the sample using a magnet rack. 3. Add the antibody conjugated acceptor and incubate them with continuous rotation for a sufficient time. 4. Analysis of numerous microplate readers support TR-FRET measurements.

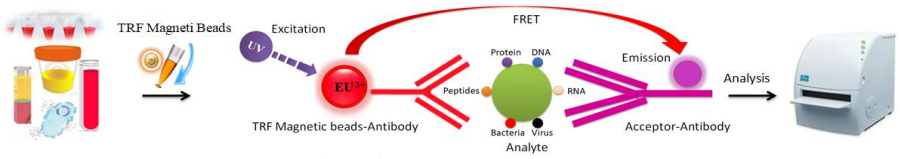

Fig.2 Workflow of TR-FRET Magnetic Beads Assay

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Advantages and benefits

1. Perform a double function simultaneously on the same beads: The magnetic beads combine separation/preconcentration and detect analytes, allowing quick, simple, robust, and high-throughput analytes of trace amounts from complex biological samples on the same beads.
2. Ultra sensitive. Lower detection limits of 10 pg/mL versus typical fluorometric detection limits of 100 pg/mL
3. Extremely photostable and highly resistant to photobleaching. All the lanthanide chelate or cryptate molecules and iron oxide are entirely encapsulated inside each bead instead of merely on the bead's surface. The protective environment prevents iron oxide and dye from leaching into aqueous media, which makes the beads less sensitive to external conditions such as solvent, temperature, pH, etc.
4. Very high fluorescent intensity. Because a single bead has a large concentration of lanthanide chelate with a high quantum yield ranging from 40 to 90%, the beads show excellent fluorescence intensity, which increases test sensitivity without signal amplification. Such bright beads are also perfect for donors' use in time-resolved FRET assays.
5. Lanthanide chelate or cryptate has large Stokes shifts (>250 nm), narrow emission bands (-10 nm bandwidth), and long fluorescence lifetime (μs), which dramatically reduces background and increases the signal-to-noise ratio.
6. Most bioprocess ELISA assays can be converted to an HTRF assay.
7. No washing step is involved in the assays.
8. Have a hydrophilic silica surface grafted by different functional groups with linkers of variable lengths, allowing efficient conjugation of various ligands such as peptides, protein, antibodies, small molecules, carbohydrates, aptamers, DNA/RNA, etc.
9. Due to the microsphere's magnetic property, the fluorescent magnetic beads are suitable for high-throughput automation.

Related Products	
Streptavidin Europium Fluorescent Magnetic Beads	Aldehyde-Activated Europium Fluorescent Magnetic Beads
Streptavidin Terbium Fluorescent Magnetic Beads	Aldehyde-Activated Terbium Fluorescent Magnetic Beads
Streptavidin-Ruthenium Fluorescent Magnetic Beads	Aldehyde-Activated Ruthenium Fluorescent Magnetic Beads
Avidin Europium Fluorescent Magnetic Beads	Amine Activated-Europium Fluorescent Magnetic Beads
Avidin Terbium Fluorescent Magnetic Beads	Amine-Activated Terbium Fluorescent Magnetic Beads
Avidin Ruthenium Fluorescent Magnetic Beads	Amine-Activated Ruthenium Fluorescent Magnetic Beads
Protein A and G Europium Fluorescent Magnetic Beads	Carboxyl-Activated Europium Fluorescent Magnetic Beads
Protein A and G Terbium Fluorescent Magnetic Beads	Carboxyl-Activated Terbium Fluorescent Magnetic Beads
Protein A and G Ruthenium Fluorescent Magnetic Beads	Carboxyl-Activated Ruthenium Fluorescent Magnetic Beads
Protein A Europium Fluorescent Magnetic Beads	Hydrazide-Activated Europium Fluorescent Magnetic Beads
Protein A Terbium Fluorescent Magnetic Beads	Hydrazide-Activated Terbium Fluorescent Magnetic Beads
Protein A Ruthenium Fluorescent Magnetic Beads	Hydrazide-Activated Ruthenium Fluorescent Magnetic Beads
Protein G Europium Fluorescent Magnetic Beads	Iodoacetyl-Activated Europium Fluorescent Magnetic Beads
Protein G Terbium Fluorescent Magnetic Beads	Iodoacetyl-Activated Terbium Fluorescent Magnetic Beads
Protein G Ruthenium Fluorescent Magnetic Beads	Iodoacetyl-Activated Ruthenium Fluorescent Magnetic Beads
Protein L Europium Fluorescent Magnetic Beads	NHS-Activated Europium Fluorescent Magnetic Beads
Protein L Terbium Fluorescent Magnetic Beads	NHS-Activated Terbium Fluorescent Magnetic Beads
Protein L-Ruthenium Fluorescent Magnetic Beads	NHS-Activated Ruthenium Fluorescent Magnetic Beads

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