Iodoacetyl-activated Terbium Fluorescence Magnetic Beads

BcMag™ Iodoacetyl-activated Terbium Fluorescence Magnetic Beads (Fig.1) are Time-Resolved Fluorescence (TRF) magnetic microspheres coated with a high density of Iodoacetyl functional groups on the surface. The microspheres combine the benefits of the preactivated Iodoacetyl active group, time-resolved Fluorescence dyes, and magnetic characteristics to perform very sensitive assays. The beads are manufactured using nanometer-scale superparamagnetic iron oxide and europium metal as core and entirely encapsulated by a high-purity silica shell, ensuring no leaching problems with the iron oxide and europium metal.

Fig.1 BcMag™ Terbium Fluorescence Magnetic Beads

It is designed to enable fast, efficient, and covalent immobilization of proteins, peptides, and other ligands through their sulfhydryl groups (-SH) for affinity purification procedures. At physiological to alkaline circumstances (pH 7.2 to 9)) in either aqueous or organic solvents with 20- 30% DMSO or DMF, iodoacetyl-activated supports react with sulfhydryl groups, resulting in stable thioether bonds. These reactions are often carried out in the dark to prevent the formation of free iodine, which can react with tyrosine, histidine, and tryptophan residues. The hydrophilic surface ensures beads low nonspecific adsorption, excellent dispersion, and easy handling in various buffers.

It is frequently helpful to immobilize affinity ligands via functional groups other than amines. The thiol group, in particular, can be employed to guide coupling processes away from active centers or binding sites on specific protein molecules.

Cysteines are frequently bonded between their side chains via disulfide bonds (-S-S-) as part of a protein's secondary or tertiary structure. The side chain of cysteine contains sulfhydryls (-SH) (Cys, C). These must be converted to sulfhydryls before being immobilized. Thiol groups (sulfhydryls) can be found naturally in proteins or introduced through the reduction of disulfides or using various thiolation reagents.

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.

Fluorescence dye properties

Fluorophore	Fluorescence color	Excitation (nm)	Emission (nm)	Fluorescence lifetime (τ) (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	~1.47 x 10^8 beads/mg (2.5μm beads) ~ 5 x 10^7 beads /mg (5μm beads)

Phone: +1-858-909-0079 **Bioclone Inc**. Fax: +1-858-909-0057

Stability	Short Term (<1 hour): pH 3-11; Long-Term: pH 4-10 Temperature: 4°C -140°C; Most organic solvents	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Formulation	Lyophilized Powder	
Functional Group Density	2.5µm Magnetic Beads	~195 µmole / g of Beads
	5µm Magnetic Beads	~180 µmole / g of Beads
Storage	Store at -20°C upon receipt.	

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

BcMag™ TR-FRET Assay, in contrast to typical FRET (Förster Resonance Energy Transfer) assays, uses time-resolved Fluorescence magnetic beads (BcMag™ TR-Magnetic Beads) as the donor fluorophore. The donor and acceptor can be two proteins, two DNA strands, an antigen, 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 Fluorescence 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 Fluorescence dyes. BcMag™ Iodoacetyl-activated Terbium Fluorescence 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 supports TR-FRET measurements.

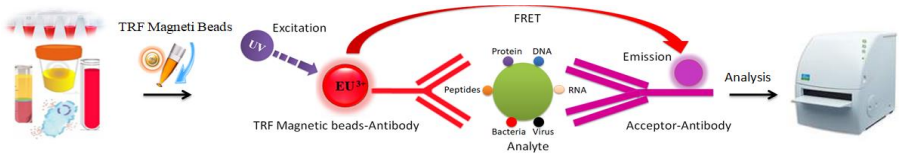

Fig.2 Workflow of TR-FRET Magnetic Beads Assay

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 Fluorescence 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, proteins, antibodies, small molecules, carbohydrates, aptamers, DNA/RNA, etc.
9. Due to the microsphere's magnetic properties, the Fluorescence magnetic beads are suitable for high-throughput automation.

Conjugation protocol

Iodoacetyl-Activated Magnetic Beads

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