

# Luminspheres™

## 4-Colour Bioconjugation Kit Protocol

### Product Information

**Product Code** LUM001

**Batch Number** See label on vials

**Storage** This kit should be stored at room temperature in the dark and used within 6 months.

**Bench-life** Reconstituted Luminspheres™ should be conjugated to the biomolecule immediately.

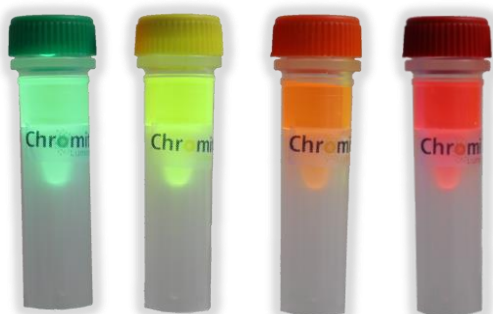
**Use** Research use only. Not for use in diagnostic procedures.

**Applications** Fluorescent immunohistochemistry, cytometry, western blot, lateral flow and ELISA.

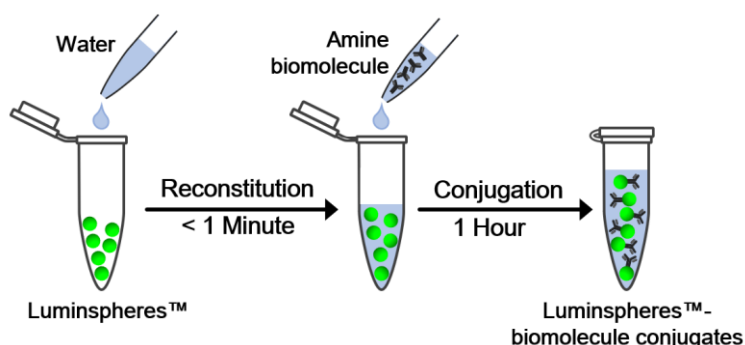
**Safety Note** The hazards associated with Luminspheres™ are classified as unknown. All other reagents in the kit are classified as non-hazardous. It is strongly recommended to wear disposable gloves, laboratory coat and safety glasses while working with the items in this kit.

### Luminspheres™ Bioconjugation Kit Description

Luminspheres™ 4-Colour Bioconjugation Kit enables multiplex imaging of biological systems using techniques such as immunohistochemistry, cytometry, western blot, cell imaging, lateral flow and ELISA. Luminspheres™ are extraordinary bright fluorophores, several orders of magnitude brighter than molecular systems, allowing biomolecules labelled with Luminspheres™ to be imaged at exceptional sensitivity. Multicoloured Luminspheres™ can be visualised simultaneously when irradiated with a single excitation source (UVA/violet light); without complex imaging hardware such as multiple light sources and filters sets. Luminspheres™-biomolecule conjugates are prepared using this kit by coupling to amino-functional biomolecules without the need for the addition of coupling reagents.



Luminspheres™ 4-Colour Bioconjugation Kit



Luminspheres™ Bioconjugation Procedure

### Reagents and Equipment

#### Kit Contents

- 100 µg of freeze-dried green Luminspheres™
- 100 µg of freeze-dried yellow Luminspheres™
- 100 µg of freeze-dried orange Luminspheres™
- 100 µg of freeze-dried red Luminspheres™
- 1 mL 40x conjugation buffer
- 1 mL 1x quench buffer

#### Required Reagents and Equipment

- Ultra-pure deionised water
- Amine functionalised biomolecule
- 15 mL centrifuge tubes
- 100-1000 µL pipette tips
- Variable volume pipettes (100-1000 µL).
- Laboratory tube rotator

# Luminspheres™

## 4-Colour Bioconjugation Kit Protocol

### Example Bioconjugation Protocol: Luminspheres™-Goat-*anti*-Rabbit Antibody Conjugates

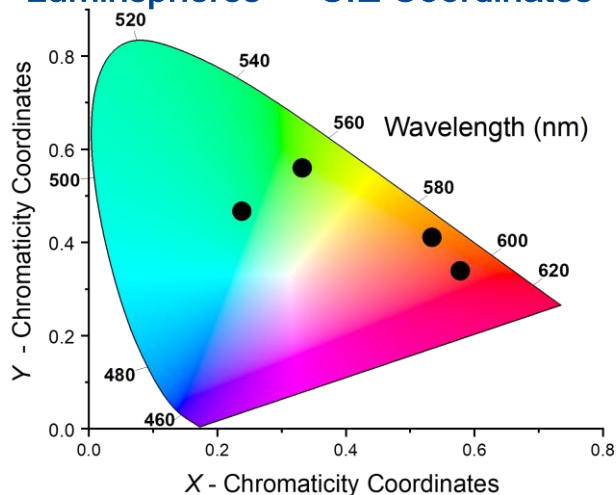
*Optimisation of this procedure is required for conjugating your desired amine functionalised biomolecule*

1. The Luminspheres™ kit should be stored at room temperature in the dark.
2. Pipette 100 µL of deionized water into a Luminspheres™ vial and agitate until the Luminspheres™ are fully dispersed in the water. (*Luminspheres™ concentration is 1 mg mL<sup>-1</sup>*).
3. Prepare 1x conjugation buffer by diluting the 1 mL 40x conjugation buffer with 39 mL of ultrapure deionised water.
4. Transfer 50 µL of the dispersed Luminspheres™ solution to 4.90 mL of the 1x conjugation buffer and agitate until homogenous.
5. Immediately add 50 µg of the goat-*anti*-rabbit antibody (*the mass ratio of Luminspheres™ to antibody is 1:1*).
6. Mix the sample for 1 hour on a laboratory tube rotator.
7. Add 50 µL of the 1x quench buffer and mix the sample for 1 hour on a laboratory tube rotator.
8. To check that the conjugation has been successful, run two separate protein A/G lateral flow assays (kit available from Abcam with product code ab236554) of the unconjugated Luminspheres™ and the Luminspheres™-antibody conjugates. For lateral flow the Luminspheres™ and their conjugates should be prepared by diluting 10 µL in 90 µL of the running buffer. *Positive binding of the Luminspheres™-antibody conjugates to the lateral flow strip indicates a successful conjugation between Luminspheres™ and antibody, the unconjugated Luminspheres™ should not bind to the lateral flow strip.*
9. Keep Luminspheres™-antibody conjugates at 4°C and it is recommend they are used immediately
10. Removal of any unconjugated antibody from the Luminspheres™-antibody conjugates can be achieved by transferring the conjugates (5 mL) to dialysis tubing with a MWCO of 1,000 KDa and diameter 10 mm (available from Repligen, product code: 131486T). Dialysis should be conducted against pH 7.4 25 mM HEPES buffer (500 mL) for 20 hours at room temperature in the dark.

### Luminspheres™ Optical Properties

	Green	Yellow	Orange	Red
<b>Excitation Light Source</b>	UVA to violet light (360-410 nm)			
<b>Colour Emission Maxima (± 3 nm)</b>	514	534	598	627
<b>CIE Coordinates (x, y)</b>	0.238, 0.467	0.332, 0.560	0.534, 0.411	0.578, 0.339

### Luminspheres™ CIE Coordinates



### Luminspheres™ Emission Spectra

