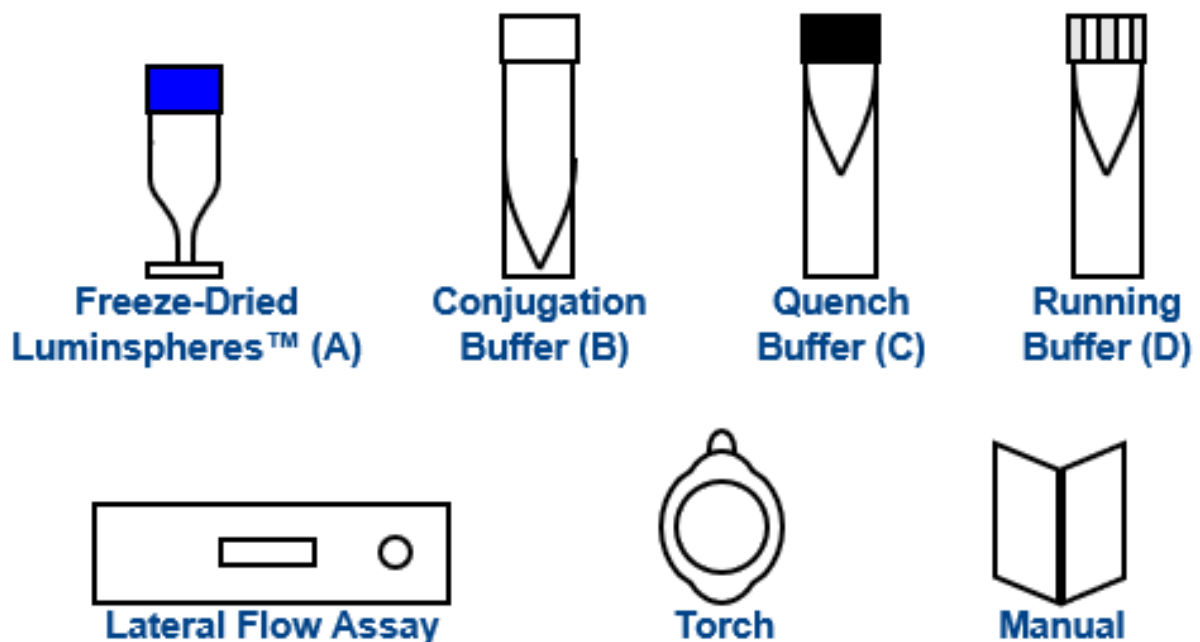


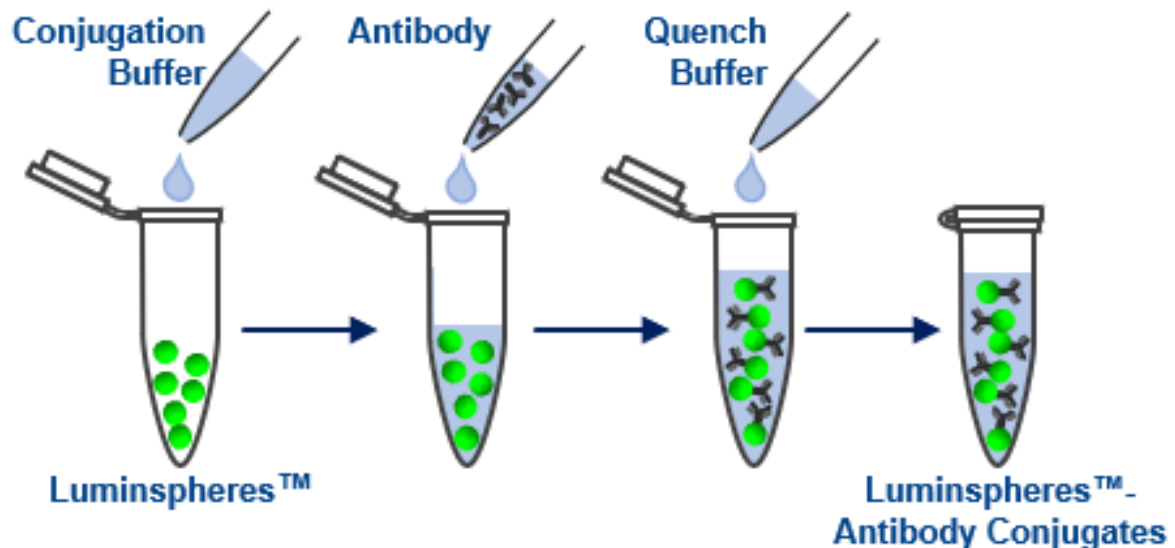
## Freeze-Dried Luminspheres™ Bioconjugation Kit

<b>Product Code</b>	CHRLUMG01
<b>Lot Number</b>	See individual contents
<b>Storage</b>	This kit should be stored at 2-8 °C in the dark.
<b>Use</b>	Research use only. Not for use in diagnostic procedures.
<b>Applications</b>	Immunofluorescence
<b>Safety Note</b>	The hazards associated with Luminspheres™ are classified as unknown. All other reagents in the kit are classified as non-hazardous. Wear disposable gloves, laboratory coat and safety glasses.
<b>Disposal</b>	Dispose of in accordance with Hazardous Waste Regulations 2005 and any additional local regulations.

### Kit Contents



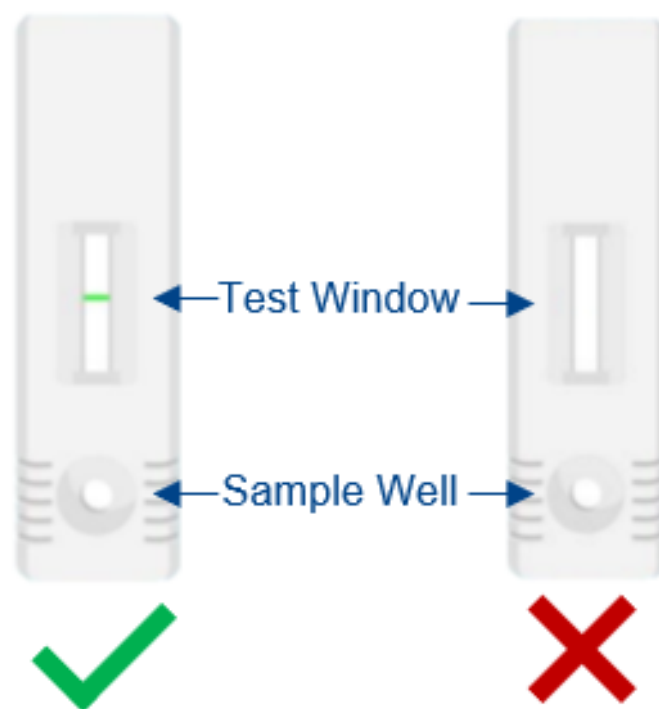
## Antibody Conjugation Protocol



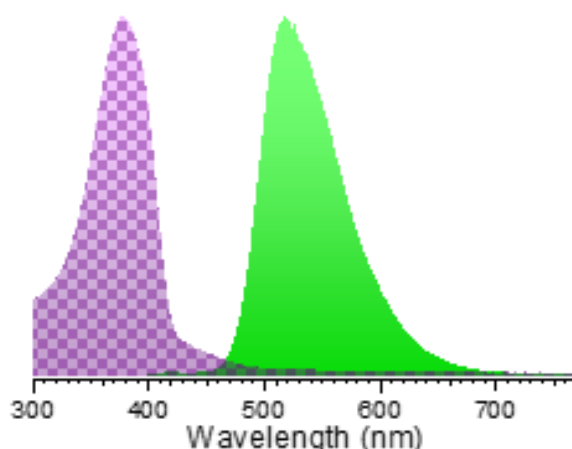
1. Before use, centrifuge (1,000 rpm, 30 seconds); **Conjugation Buffer (B)**, **Quench Buffer (C)** and **Running Buffer (D)** to concentrate solutions to bottom of the tube.
2. Dissolve the **Freeze-Dried Luminspheres™ (A)** by adding all the **Conjugation Buffer (B)** (940  $\mu$ L) by repeatedly aspirating and expelling from the pipette tip.
3. Immediately add 10  $\mu$ g of the required antibody (*not supplied, free from additional proteins, azide and amine containing buffers*) and rotate (10-20 rpm) for 1 hour at room temperature in the dark.
4. Add all the **Quench Buffer (C)** (50  $\mu$ L) and rotate (10-20 rpm) for 1 hour at room temperature in the dark.
5. Use the Luminspheres™-antibody conjugates immediately or store at 4  $^{\circ}$ C, in the dark and use within 1 week.

## Antibody Conjugation Confirmation Test

1. Add the prepared Luminspheres™-antibody conjugates (10 µL) to all the **Running Buffer (D)** (90 µL).
2. Transfer all this solution (100 µL) to the 'Sample Well' of the **Lateral Flow Assay**.
3. Leave the **Lateral Flow Assay** to run for 30 minutes.
4. Illuminate the 'Test Window' using the supplied **Torch**.
5. A single green fluorescent line indicates successful conjugation between antibody and Luminspheres™. If no fluorescent line present; check for aggregation and repeat with different antibody.



## Luminspheres™ Excitation & Emission Spectra



### Compatible Instruments

Recommended instruments and settings, this list is not exhaustive.

#### Fluorescence Microscopy

##### Nikon

UV-2A (BP 355/50, BS 400, LP 410)  
V-2A (BP 400/40, BS 430, LP 440)

##### Leica

Filter cube A (BP 360/40, BS 400, LP 425)  
Filter cube D (BP 355-425 BS 455, LP 470)

##### Olympus

U-MWU (BP 330-385, BS 400, LP 420)

##### Zeiss

Filter Set 02 (BP 365, BS 395, LP 420)

#### Microplate Reader

##### Glomax explorer (Promega)

Excitation wavelength: 365nm  
Detection region: 500–550nm

##### CLARIOstar (BMG LABTECH)

Excitation wavelength: 385/30 nm  
Detection region: 525/30 nm

##### SpectraMax i3x (Molecular Devices)

Excitation wavelength: 385/30 nm  
Detection region: 525/30 nm

##### Fluoroskan (ThermoFisher)

Excitation wavelength: 380/11 nm  
Detection region: 518/13 nm

#### Flow Cytometry

##### ZE5 Cell Analyzer (Biorad)

Excitation laser: 405 nm  
Detection filters: 525/50 and LP 439, 493 nm

##### CytoFLEX (Beckman)

Excitation laser: 375 and 405 nm  
Detection filter: BP 525/40 nm

##### FAC Symphony (BD Biosciences)

Excitation laser: 375 and 405 nm  
Detection filters: BP 515/20 or 525/40 nm

##### Attune (ThermoFisher)

Excitation laser: 375 and 405 nm  
Detection filters: BP 512/25 or 530/30 nm