



### KIT DESCRIPTION

This kit is based on our lanthanide nanoparticle technology, which offers outstanding performance in lateral flow immunoassays (LFIA). The Link-Dtech™ reagent is intended for the coupling of antibodies or proteins to Link-Dtech™ nanoparticles. Detection is performed using Time-Resolved Fluorescence (TRF). Excitation and emission maxima (nm): [see table 1 on page 2](#).

#### Supplied reagents and storage

|                                               |                |
|-----------------------------------------------|----------------|
| <b>Link-Dtech™</b><br>( $1 \times 10^{-8}$ M) | Store at 2-8°C |
| <b>Coupling buffer</b>                        | Store at 2-8°C |
| <b>Migration buffer</b>                       | Store at 2-8°C |



### MATERIALS AND EQUIPMENT REQUIRED (NOT INCLUDED)

- Biological entity (antibody, protein, analyte).
- Purification / desalting column (optional).
- Glass tubes with cap or low-protein-binding tubes.
- Precision micropipettes with disposable tips.
- Ultrasonic bath or vortex.
- UV lamp.
- Rotating shaker.
- Plate reader with TRF option.

### RECOMMENDED WORKING SOLUTION PREPARATION

Please watch the video “Link-Dtech – How to Use ?” on YouTube: <https://youtu.be/21HC5tL9Mv4>

#### 1) Protein preparation and calculation of the coupling ratio

- The protein to be conjugated must be prepared in a buffer compatible with the Link-Dtech™ coupling procedure. The protein solution should be free of phosphate ions, ammonium ions, primary amines, sodium azide, and BSA, as these components may interfere with the coupling reaction. **PBS is not recommended for use with Link-Dtech™.**

If the protein is supplied in an incompatible buffer, such as PBS, Tris or glycine, the buffer should be exchanged for a HEPES-based buffer before starting the conjugation procedure.

Buffer exchange can be performed using, for example:

- Zeba™ Spin Desalting Columns
- Amicon® Ultra Centrifugal Filters”

- Calculate the amount of protein to be coupled to the nanoparticle based on its molar concentration.

For antibodies, we recommend starting with a coupling ratio of 5-150 antibodies per nanoparticle. The optimal antibody-to-nanoparticle ratio should be determined according to the intended application, desired assay performance, and detection instrument. To do this, first, determine the molar concentration of the antibody (A):

$$[\text{Antibody (mol/L)}] = \frac{[\text{Mass (g/L)}]}{\text{Molecular weight (g/mol)}}$$

Next, select the volume of Link-Dtech™ nanoparticles to be used. A volume more than 20 µL is recommended to minimize variability associated with subsequent centrifugation steps. The amount of Link-Dtech™ nanoparticles (n) can then be calculated as follows (B):

$$n_{\text{Link-Dtech (mol)}} = [\text{Link-Dtech (mol/L)}] \times V_{\text{Link-Dtech (L)}}$$

Finally, calculate the volume of antibody required to obtain the desired antibody-to-nanoparticle ratio (C):

$$V_{\text{Antibody (L)}} = \frac{n_{\text{Link-Dtech (mol)}}}{[\text{Antibody (mol/L)}]} \times \text{number of antibodies to be coupled on Link-Dtech}$$

2) Antibody/Link-Dtech conjugation

2.1 Coupling step

- 1. Sonicate the Link-Dtech™ nanoparticles for 1 minute at room temperature.
- 2. Prepare the antibody/Link-Dtech conjugation mixture in a low-protein-binding tube by diluting the required volume of Link-Dtech™ (previously sonicated) in 500-1500 µL of Coupling Buffer. Mix by vortexing.
- 3. Add the volume of antibody calculated in Section 1.2.C. *Note: Add the components in the following order: Link-Dtech™ + Coupling Buffer + Antibody*
- 4. Incubate overnight at 4°C under gentle rotation.

2.2 Purification step (after overnight incubation)

- 1. Centrifuge the conjugation mixture at 10,000 x g for 30 minutes at 4-10°C.
- 2. Carefully remove the supernatant containing the unbound or excess antibody without disturbing the nanoparticle pellet.
- 3. Resuspend the pellet in Coupling Buffer to obtain a final concentration of 1x10<sup>-8</sup> M.
- 4. Sonicate until the conjugate is fully resuspended.

This conjugate may be stored at 4°C for up to 6 months.

**Technical information:** This method and the buffers provided are intended only as a general guideline. Optimal conjugation procedures are protein-dependent; optimization techniques and buffer formulations may differ for each protein/assay.

This kit is for **Research Use Only**, not for diagnostic procedures.

3) Working solution preparation

The working solution should be prepared freshly before each use.

- 1. Resuspend the antibody-conjugated Link-Dtech™ nanoparticles by thoroughly mixing the tube by sonication (recommended) or vigorous vortexing.
- 2. In a glass tube or a low-protein-binding tube, dilute the antibody-conjugated Link-Dtech™ nanoparticles in the Migration Buffer according to the recommended concentrations provided in the table below:

| Application | Link-Dtech concentration*                     | Dilution factor |
|-------------|-----------------------------------------------|-----------------|
| LFIA        | [1x10 <sup>-10</sup> – 1x10 <sup>-9</sup> M]  | 1/100 – 1/10    |
| FLISA/FRET  | [1x10 <sup>-11</sup> – 2x10 <sup>-10</sup> M] | 1/1000 – 1/50   |
| Dot-Blot    | [1x10 <sup>-11</sup> – 1x10 <sup>-9</sup> M]  | 1/1000 – 1/10   |

\*Reader dependent. Optimal concentrations/dilutions should be determined by the end user.

- 3. Close the tube tightly.
- 4. Sonicate for 1 minute at 4-15 °C. Alternatively, vortex for 1 minute at room temperature.

The antibody-conjugated Link-Dtech™ working solution is now **ready to use**. For optimal performance, use the working solution immediately after preparation.

TABLE 1. Link-Dtech™ Lanthanide Nanoparticle Specifications and Product Information

| REFERENCE | DESCRIPTION                    | EX/EM*<br>(nm)           | VOLUME | COUPLING<br>BUFFER | MIGRATION<br>BUFFER |
|-----------|--------------------------------|--------------------------|--------|--------------------|---------------------|
| LDT545    | Link-Dtech 545 – Tb (Green)    | 340 / 545                | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |
| LDT595    | Link-Dtech 595 – Dy (Yellow)   | 340 / 575<br>(480)       | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |
| LDT600    | Link-Dtech 600 – Sm (Orange)   | 340 / 600<br>(650)       | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |
| LDT614    | Link-Dtech 614 – Eu (Red)      | 340 / 614<br>(595 - 693) | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |
| LDT880    | Link-Dtech 880 – Nd (Infrared) | 340 / 880<br>(1060)      | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |
| LDT980    | Link-Dtech 980 – Yt (Infrared) | 340 / 980                | 200 µL | 25 mL              | 50 mL               |
|           |                                |                          | 2 mL   | 25 mL              | 2 x 50 mL           |
|           |                                |                          | 8 mL   | 25 mL              | 4 x 50 mL           |

\*Excitation and emission maxima in nanometers