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Product information:

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d2 labeling kit

For research use only.
Not for use in therapeutic or diagnostic procedures.

Packaging, shipping & storage details:

		Storage upon receipt
Set sent in dry ice	8 nmoles d2 dye	-60°C or below
Set sent at room temperature (RT)	Buffer and column	2-8°C

1. Kit description and intended use

The HTRF d2 labeling kit is a comprehensive set of reagents that enables the covalent coupling of d2 acceptor dye to biomolecules.

The kit is suitable for labeling peptides, proteins, oligonucleotides. N-hydroxysuccinimide-activated d2 dye reacts with primary amines to form stable dye-conjugates under mild conditions.

d2 acceptor, an organic motif of approximately 800 Da, is highly compatible with HTRF cryptates. Its excitation spectrum overlaps that of Eu3+ and Lumi4™-Tb cryptate emission, allowing the donor to excite d2 with a high quantum yield. d2's maximum light output is at 665nm.

Note: The kit was designed as a tool for assessing the biological activity of d2-labeled moieties. For any enquiry about custom labeling services, please directly contact Cisbio Bioassays.

2. Equipment and materials required but not included

- Precision micropipettes with disposable tips, capable of dispensing 10-1000 µL.
- Column stand and clamp.
- Vortex.
- Test tubes.
- HTRF® compatible reader (more information about compatible reader at http://www.htrf.com/technology/htrfmeasurement/compatible_readers/).

3. Supplied reagents and stability

3.1 Supplied reagents

d2 labeling reagent 8 nmoles (6µg) - dessicated	1 microtube + desiccant	Store at -60°C or below until use
Purification column	1 column	Store at 2-8°C
Elution buffer (100 mM phosphate buffer pH7, 2 mM NaN_3)	1 vial, 20 mL	Store at 2-8°C until use

3.2 Reagent stability

The d2 dye should be stored at -60°C or below, the other 2 components must be stored at 2-8°C before use. Under these conditions, the kit is stable until the expiry date indicated on the box label.

4. Protocol

4.1. Protein preparation and labeling conditions

The protein to be labeled should be conditioned in 50 mM Carbonate buffer pH 9.0, by dialysis or other buffer exchange procedure. The labeling process is pH and concentration sensitive. Make sure that the pH of the buffer is 9.0 and that the concentration of the protein is at 16.67 µM (e.g. 2.5 mg/mL for an antibody).

Depending on the molecular weight of the target, each kit enables the labeling of 25 µg to 250 µg (15 kDa to 150 kDa MW respectively) of molecule with an initial molar ratio of 5 d2 per molecule.

Concentration of the molecule to be labeled should be determined at its maximum absorption using the corresponding molar extinction coefficient, i.e. for an antibody :

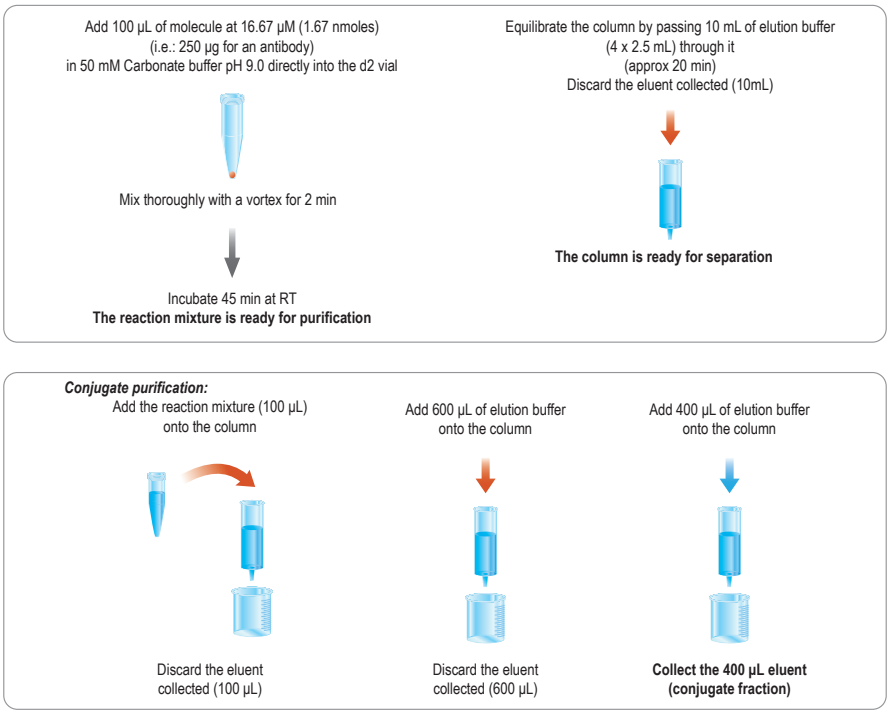
$$[\text{antibody}]\ \text{mole/L} = \frac{OD_{280nm}}{210{,}000}$$

• Where 210,000 is the molar extinction coefficient (ε) in cm-1M-1 of a typical IgG at 280nm.

4.2. Labeling procedure

The complete procedure is described alongside and takes approximately 1h.

The column supplied with this kit cannot dry out. The volume of eluent recovered at each step corresponds exactly to the volume loaded. Wait until the elution of each step is completed before starting the next one.



4.3. Determination of the degree of labeling

Measure the absorbance of the purified conjugate in a cuvette with 1cm pathlength at both 280nm (OD_{280nm}) and 650nm (OD_{650nm}).

4.3.1. Determination of the concentration of molecule labeled in the sample (example of an Ab):

$$\text{Molecule concentration (M)} = \frac{OD_{280nm} - (OD_{650nm}/28)}{210{,}000}$$

• Where 210,000 is the molar extinction coefficient (ε) in cm-1M-1 of a typical IgG at 280nm and 28 a correction factor for the d2's contribution to the absorbance at 280nm.

• The massic (mg/ml) extinction coefficient (ε) in cm-1M-1 of a typical IgG at 280nm is 1.4 and MW is 150,000.

4.3.2. Determination of the final concentration of dye in the sample:

$$\text{Dye concentration (M)} = \frac{OD_{650nm}}{250{,}000}$$

• Where 250,000 is the molar extinction coefficient (ε) in cm-1M-1 of the d2 dye at 650nm

4.3.3. Determination of the final molecular ratio (MRf) between molecule and dye in the conjugate:

$$\text{Moles of d2 per mole of molecule (MRf)} = \frac{\text{Dye concentration (M)}}{\text{Molecule concentration (M)}}$$

In general, for IgG, the labeling procedure allows 70% of initial material to be recovered and we find that labeling with 2 to 4 moles of d2 per mole of antibody is optimal.

4.4. Preparation of the conjugate stock solution

Prepare the conjugate stock solution by adding 0.1% Tween 20 and when possible 0.1% BSA to the conjugate fraction recovered, e.g. to the 400 µL conjugate fraction add 4 µL of a 10% Tween 20 solution and 4 µL of a 100 mg/mL BSA solution. Divide the conjugate into aliquots and freeze at –20°C.

4.5. Conjugate Working concentration recommendation

Conjugate working concentration will depend on the assay considered. For a d2-labeled antibody, in 384-well low volume plate format, 20ng of antibody per well (i.e. 0.13 pmol/well) may be used as a starting point. Further assay optimization will require the conjugate titration by assessing a range of antibody concentrations ranging from 1ng to 100 ng per well.

4.6. Conjugate storage conditions and handling

Divide the stock solution into suitably sized aliquots and store at -20°C. Avoid repeated thaw/freeze cycles. Most common buffers can be used for the preparation of the working solution, providing that the pH is maintained between 5.5 and 8.5. They need to be complemented with BSA. (0.1%) to prevent reagent coating, and detergents such as Tween 20, Triton X100 or CHAPS (up to 0.5%) may also be added.

4.7. Recommendations

- Always store the d2 under a desiccated atmosphere. d2 is stable for 3 months at -60°C or below.
- Strictly follow the instructions. Always start working with a 5-fold amount of d2 per molecule to be labeled to ensure an efficient coupling (labeling efficacy is concentration dependent).
- The molecule to be labeled must be in a buffer free of ammonium ions or primary amines, as they will compete with the amine groups of the protein for the reactive dye.
- If the molecule is in Tris or glycine buffer or purified with ammonium sulphate, the buffer needs to be replaced with phosphate-buffered saline (PBS).
- Impure molecules or material stabilized with bovine serum albumin (BSA) or other carrier proteins cannot be labeled reliably, unless pre-purified.
- Do not elute more than 400 µL of conjugate fraction (step 4).
- Do not re-use the column.