



Magic Fast™ Cy3 Conjugation Kit

CD4004-1 mg

(User Reference Guide)

Important Notes

The information provided in this document and the methods included in this package are for information purposes only. Creative Diagnostics provides no warranty of performance or suitability for the purpose described here in. The performance of labeling using this kit may be affected by antibodies. Sample data are provided for illustration and example purposes only. Information about the chemicals and reagents used in the kit are provided as necessary.

These reagents provided are for research use only. Not for use in diagnostic procedure.

Product Overview

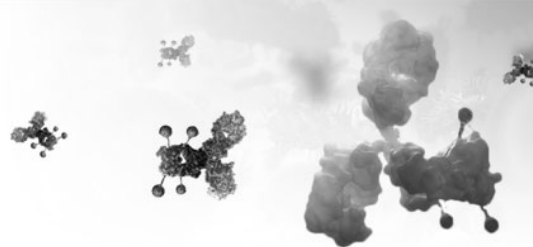
Creative Diagnostics has designed the Magic Fast™ Cy3 conjugation kit to enable the direct labeling of any proteins, antibodies, antibody fragments, peptides or other biomolecules with available RNH₂ to accelerate life science fundamental research and the development of the diagnostic kit. Our technology can be used to label both small and large quantities of biomolecules with ease. The researcher simply pipettes their materials into the vial and incubates for 0.5 hours, the whole conjugation process takes less than 1 hour. The labelled antibodies or proteins can be used for ELISA, Western blot, IF and FACS analysis.

Performance

- Material selection: Any proteins, antibodies, antibody fragments, peptides or other biomolecules with available RNH₂.
- Reactivity: RNH₂.
- Linkage stability: Activation of proprietary reagents within the offered solution results in directional covalent bonding of Cy3 to the biomolecules.
- Easy-to-go: Instant reaction; the whole process is about 1 hour.
- High purity: The product after desalting has a high purity.
- Consistency: Good reproducibility from batch to batch.

Kit Components

Cat. No	Cy3 Powder	Cy3 Diluent	Conjugation Buffer	Purification Buffer	Desalting Column
CD4004-1 mg	1 mg	200 µl	5 ml	15 ml	2 Columns



Recommended Application Notes

- Cy3 Powder (mg) / Cy3 Diluent (µl): 1 mg / 100 µl
- Material (mg) / Conjugation Buffer (µl): 1 mg / 500 µl
- Mole ratio: Material / Cy3=1/20~1/30

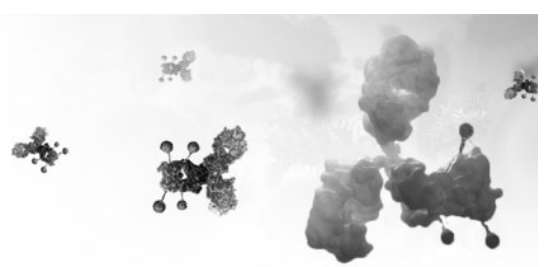
Each laboratory should determine an optimum amount for use in its particular application.

Buffer Considerations

Buffer Components & Conditions	
Amine free buffer (e.g HEPES, MES, MOPS and phosphate)	Yes
Borate buffer	Yes
Chelating agents (e.g. EDTA)	Yes
BSA	No
Glycerol	No
Glycine	No
Material supplied in ascites fluid, serum, hybridoma or tissue culture media	No
Merthiolate	No
Non-buffering salts (e.g. sodium chloride)	Yes
Other biomolecules	Primary amines required
Peptides	Yes
pH	6.5-8.5
Proclin	No
Proteins	Yes
Purified antibody	Yes
Sodium Azide	No
Sugars	Yes
Thiomersal / Thimerosal	No
Tris	No

Storage

- Cy3 Powder: Stable at 2-8°C for six months. Keep in dark place.
- Cy3 Diluent: Stable at room temperature for six months.
- Conjugation Buffer: Stable at 2-8°C for six months.
- Purification Buffer: Stable at 2-8°C for six months.
- Desalting Column: Stable at 2-8°C for six months.



Attention

- Cy3 solution should be used immediately and stored away from light.
- If the buffer of the antibody contains interfering substances such as BSA, the buffer should be changed into the conjugated buffer with an ultrafiltration tube before labeling, and the interfering components in the buffer should be removed.
- The amount of Cy3 and antibody should be calculated in advance. The volume of the desalting column resin is 3 ml, and the recommended sample volume is 0.3-0.9 ml. The sample volume should not exceed 20% of the resin volume (0.6 ml).
- The whole conjugation process should be carried out in a dark environment.
- A high F/P value will cause self-quenching of fluorescent probes and affect the biological activity of biomolecules. The absorbance coefficient of Cy3 at 552 nm is 150 000 M⁻¹cm⁻¹. The molar absorbance coefficient of the antibody at 280nm was 170 000 M⁻¹cm⁻¹. The absorption coefficient of Cy3 dye at 280 nm is 8% of that at 552 nm. A small amount of the conjugated product should be used to determine the UV-visible absorbance at 280 nm (antibody / protein) and 552 nm (Cy3), and the F/P value should be calculated according to the following formula:

$$[\text{Cy3}] = A552/150000$$

$$[\text{Antibody}] = \{A280 - (0.08 \times A552)\} / 170000$$

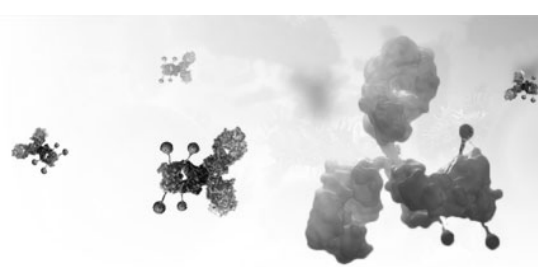
$$\text{F/P final} = [\text{Cy3}] / [\text{antibody}] = \{1.13 \times A552\} / \{A280 - (0.08 \times A552)\}$$

Preparation

1. Preparation of Cy3 solution: Add 0.1 ml of Cy3 diluent to 1 mg of Cy3 powder, mix and fully dissolve it to a final concentration of 10 mg/ml.
2. Prepare 1mg of antibody: the concentration of antibody should not be less than 2 mg/ml. If the form is powder, 1 mg antibody can be dissolved directly with 0.5 ml of coupling buffer. "If the form is liquid, the volume can be supplemented to 0.5 ml with coupling buffer."
3. Calculate the volume of Cy3 in the labeling system: the relative molar ratio of antibody to Cy3 should ideally be 1:20 to 1:30.

Assay Procedure

1. Add 10 µl of freshly prepared Cy3 solution to 1mg antibody and mix quickly.
2. Reaction at 37°C for 30min. Keep in dark place.
3. Mark one end of the desalting column and ensure that the mark is always facing outward during centrifugation.
4. Centrifuge the desalting column at 1000 g for 1 min to compact the resin.
- 5 Remove the top and bottom caps of the column, place the column into a 15 ml centrifuge tube, and centrifuge at 1000 g for 2 min to remove the storage buffer.
6. Add 2ml purification buffer to the column and centrifuged at 1000 ×g for 2min to remove the buffer.



7. Repeat the previous step five times, discard the liquid after each centrifugation.
8. Put the purification column into a new centrifuge tube, remove the lid, slowly add the conjugated product to the resin of the purification column, centrifuged at 1000 ×g for 6 min to collect the solution in the centrifuge tube, which is the purified Cy3 conjugated product.
9. The purified Cy3 conjugated product solution can be stored at 4°C in the dark for a short period of time. For long-term storage, it should be lyophilized or stored at -20°C in the dark after adding 40% glycerol. Avoid repeated freezing and thawing.

Note: The kit above is designed for antibodies and proteins.