

## Transferrin Conjugates

**Table 1.** Contents and Storage Information.

Material	Amount	Storage	Stability
Transferrin conjugates	5 mg protein, lyophilized in phosphate-buffered saline (PBS), pH 7.2† lyophilized power	<ul style="list-style-type: none"> <li>• ≤−20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	1 year

\* Degree of substitution is indicated on the product's label. † Each vial contains 15 mg of solid, including salt, and 5 mg of the transferrin conjugate.

**Approximate Fluorescence Excitation and Emission, in nm:** See Table 2.

## Introduction

Transferrin is a monomeric serum glycoprotein (~80,000 daltons) that binds up to two Fe<sup>3+</sup> atoms for delivery to vertebrate cells through receptor-mediated endocytosis.<sup>1-3</sup> Once iron-carrying transferrin proteins are inside endosomes, the acidic environment favors dissociation of iron from the transferrin–receptor complex. Following the release of iron, the apotransferrin is recycled to the plasma membrane, where it is released from its receptor to scavenge more iron.<sup>4,5</sup> Fluorescent transferrin conjugates can therefore be used with fluorescent LDL to distinguish the lysosomally directed and recycling endosomal pathways.<sup>6,7</sup>

Molecular Probes provides researchers with a selection of biotinylated and fluorescent diferric (Fe<sup>3+</sup>) human transferrin conjugates. Fluorescently labeled transferrin has greatly aided the investigation of endocytosis.<sup>8-10</sup> For instance, BODIPY® FL dye–labeled transferrin has been used to track early endosomes in HEp-2 cells by confocal laser scanning microscopy.<sup>11</sup> The pH sensitivity of fluorescein-labeled transferrin has been exploited to investigate events occurring during endosomal acidification.<sup>12-15</sup> Fluorescent transferrin has also been used to:

- Analyze the role of the  $\gamma$ -chain of type III IgG receptors in antigen–antibody complex internalization<sup>16</sup>
- Define the nature of several mutations that affect the endosomal pathway<sup>17-19</sup>
- Demonstrate that the fungal metabolite brefeldin A induces an increase in tubulation of transferrin receptors in BHK-21 cells<sup>20</sup> and in the perikaryal–dendritic region of cultured hippocampal neurons<sup>21</sup>
- Observe receptor trafficking in living cells by confocal laser scanning microscopy and show that recycling transferrin receptors are distributed on the surface of the leading lamella in migrating fibroblasts<sup>22</sup>
- Show that the endosomal compartment of living epidermoid carcinoma cells is an extensive network of tubular cisternae<sup>23</sup>
- Measure transferrin receptor binding affinity in mammals and parasites<sup>24</sup>

## Guidelines for Use

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### Preparing Transferrin Conjugates

These conjugates should be reconstituted in 1 mL of deionized water to obtain a 5 mg/mL solution in PBS. Store solutions at 2–6°C, protected from light, and with the addition of sodium azide at a final concentration of 2 mM. DO NOT FREEZE the solutions.

### Using Conjugate Solutions

It is a good practice to centrifuge the protein conjugate solution briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed in solution, thereby reducing nonspecific background staining.

### Properties

The human holotransferrin (transferrin saturated with iron) we use to prepare the transferrin conjugates is determined to be >98% pure by SDS-polyacrylamide gel electrophoresis. Peak excitation and emission wavelengths for the fluorescent transferrin conjugates are listed in Table 2.

### Warning

The transferrin conjugates contain human transferrin. The venous blood from which the human transferrin is isolated has been tested for the presence of Hepatitis B Surface Antigen (HBsAg) and for HIV (HTLV III) antibody and was found to be negative for both. However, in accordance with good laboratory procedures, these products must be handled as if they are capable of transmitting hepatitis or other infectious agents.

**Table 2.** Transferrin\* conjugates.

Cat #	Label	Ex †	Em †
T23363	biotin-XX	NA	NA
T2871	fluorescein	494	518
T13342	Alexa Fluor® 488	495	519
T2873	BODIPY® FL	505	513
T2872	tetramethylrhodamine	555	580
T23364	Alexa Fluor® 546	556	573
T35352	Alexa Fluor® 555	555	565
T23365	Alexa Fluor® 568	578	603
T13343	Alexa Fluor® 594	590	617
T2875	Texas Red®	595	615
T23362	Alexa Fluor® 633	632	647
T23366	Alexa Fluor® 647	650	668
T35357	Alexa Fluor® 680	679	702

\* Transferrin is purified from human serum. † Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. NA = not applicable. Full spectra for these dyes are available at our website ([probes.invitrogen.com](http://probes.invitrogen.com)).

## References

1. Cell 49, 423 (1987); 2. Trends Biochem Sci 12, 350 (1987); 3. Biochimie 68, 375 (1986); 4. J Cell Biol 108, 1291 (1989); 5. Cell 37, 789 (1984); 6. J Cell Biol 121, 1257 (1993); 7. J Cell Sci 107, 2177 (1994); 8. J Cell Biol 125, 253 (1994); 9. J Cell Biol 121, 61 (1993); 10. J Biol Chem 263, 8844 (1988); 11. J Cell Biol 128, 549 (1995); 12. Biochemistry 31, 5820 (1992); 13. J Bioenerg Biomembr 23, 147 (1991); 14. J Biol Chem 266, 3469 (1991); 15. J Biol Chem 265, 6688 (1990); 16. Nature 358, 337 (1992); 17. J Cell Biol 123, 1119 (1993); 18. J Cell Biol 122, 1231 (1993); 19. J Cell Biol 122, 565 (1993); 20. J Cell Biol 118, 267 (1992); 21. J Cell Biol 122, 1207 (1993); 22. J Cell Biol 125, 1265 (1994); 23. Nature 346, 335 (1990); 24. Nature 391, 499 (1998).

## Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
T13342	transferrin from human serum, Alexa Fluor® 488 conjugate	5 mg
T23364	transferrin from human serum, Alexa Fluor® 546 conjugate	5 mg
T35352	transferrin from human serum, Alexa Fluor® 555 conjugate	5 mg
T23365	transferrin from human serum, Alexa Fluor® 568 conjugate	5 mg
T13343	transferrin from human serum, Alexa Fluor® 594 conjugate	5 mg
T23362	transferrin from human serum, Alexa Fluor® 633 conjugate	5 mg
T23366	transferrin from human serum, Alexa Fluor® 647 conjugate	5 mg
T35357	transferrin from human serum, Alexa Fluor® 680 conjugate	5 mg
T23363	transferrin from human serum, biotin-XX conjugate	5 mg
T2873	transferrin from human serum, BODIPY® FL conjugate	5 mg
T2871	transferrin from human serum, fluorescein conjugate	5 mg
T2872	transferrin from human serum, tetramethylrhodamine conjugate	5 mg
T2875	transferrin from human serum, Texas Red® conjugate	5 mg

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