

Transferrin Conjugates

Table 1 Contents and storage

Material	Formulation	Storage	Stability
Transferrin conjugates*	lyophilized powder containing transferrin conjugate, lyophilized in phosphate-buffered saline (PBS), pH 7.2	<ul style="list-style-type: none"> • ≤-20°C • Desiccate • Protect from light 	1 year

* Degree of substitution is indicated on the product's label.

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

Introduction

Transferrin is a monomeric serum glycoprotein (~80,000 daltons) that binds up to two Fe³⁺ atoms for delivery to vertebrate cells through receptor-mediated endocytosis.¹⁻³ 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.^{4,5} Fluorescent transferrin conjugates can therefore be used with fluorescent LDL to distinguish the lysosomally directed and recycling endosomal pathways.^{6,7}

Life Technologies offers researchers a selection of biotinylated and fluorescent diferric (Fe³⁺) human transferrin conjugates. Fluorescently labeled transferrin has greatly aided the investigation of endocytosis.⁸⁻¹⁰ For instance, the pHrodo™ Red conjugate is almost non-fluorescent at neutral pH, but fluoresces brightly in acidic environments (Figure 1, page 2). This property can aid in investigation of endocytosis, where the pH of endosomes is less than it is outside of cells. These and other fluorescently labeled transferrin conjugates have been used to track early endosomes in HEp-2 cells by confocal laser scanning microscopy.¹¹⁻¹⁵

Fluorescent transferrin has also been used to:

- Analyze the role of the γ -chain of type III IgG receptors in antigen–antibody complex internalization¹⁶
- Define the nature of several mutations that affect the endosomal pathway¹⁷⁻¹⁹
- Demonstrate that the fungal metabolite brefeldin A induces an increase in tubulation of transferrin receptors in BHK-21 cells²⁰ and in the perikaryal–dendritic region of cultured hippocampal neurons²¹
- 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 fibroblasts²²
- Show that the endosomal compartment of living epidermoid carcinoma cells is an extensive network of tubular cisternae²³
- Measure transferrin receptor binding affinity in mammals and parasites²⁴

For Research Use Only. Not for use in diagnostic procedures.

Table 2 Transferrin* conjugates

Label	Cat. no.	Ex †	Em †	pH response	Amount
Biotin-XX	T23363	NA	NA	No	5 mg
Fluorescein	T2871	494	518	Yes	5 mg
Alexa Fluor® 488	T13342	495	519	No	5 mg
Tetramethylrhodamine	T2872	555	580	No	5 mg
Alexa Fluor® 546	T23364	556	573	No	5 mg
Alexa Fluor® 555	T35352	555	565	No	5 mg
Alexa Fluor® 568	T23365	578	603	No	5 mg
pHrodo™ Red	P35376	560	585	Yes	1 mg
Alexa Fluor® 594	T13343	590	617	No	5 mg
Texas Red®	T2875	595	615	No	5 mg
Alexa Fluor® 633	T23362	632	647	No	5 mg
Alexa Fluor® 647	T23366	650	668	No	5 mg
Alexa Fluor® 680	T35357	679	702	No	5 mg

* Transferrin is purified from human serum. † Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. Full spectra for these dyes are available on our website at www.lifetechnologies.com. NA = not applicable.

Figure 1 The fluorescence emission spectra of the pHrodo™ Red conjugate

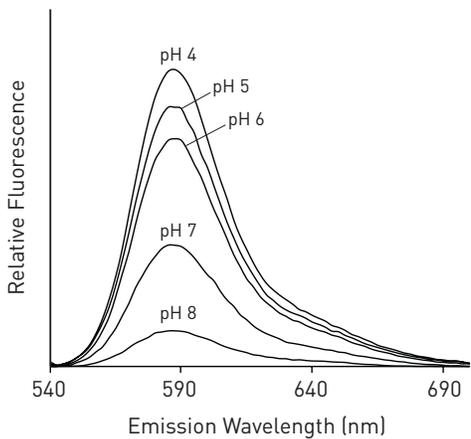


Figure 2 K562 erythroleukemia cells were loaded with either 25 µg/mL pHrodo™ Red Transferrin conjugate (dark grey area) or were left unstained as negative control (light grey area). Both samples were incubated at 37°C for 15 minutes. The red fluorescent signal rapidly increases as the transferrin conjugate is internalized into acidic compartments. Samples were acquired and analyzed using the Attune® Acoustic Cytometer.

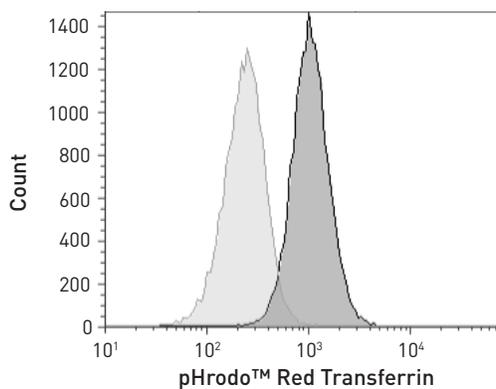
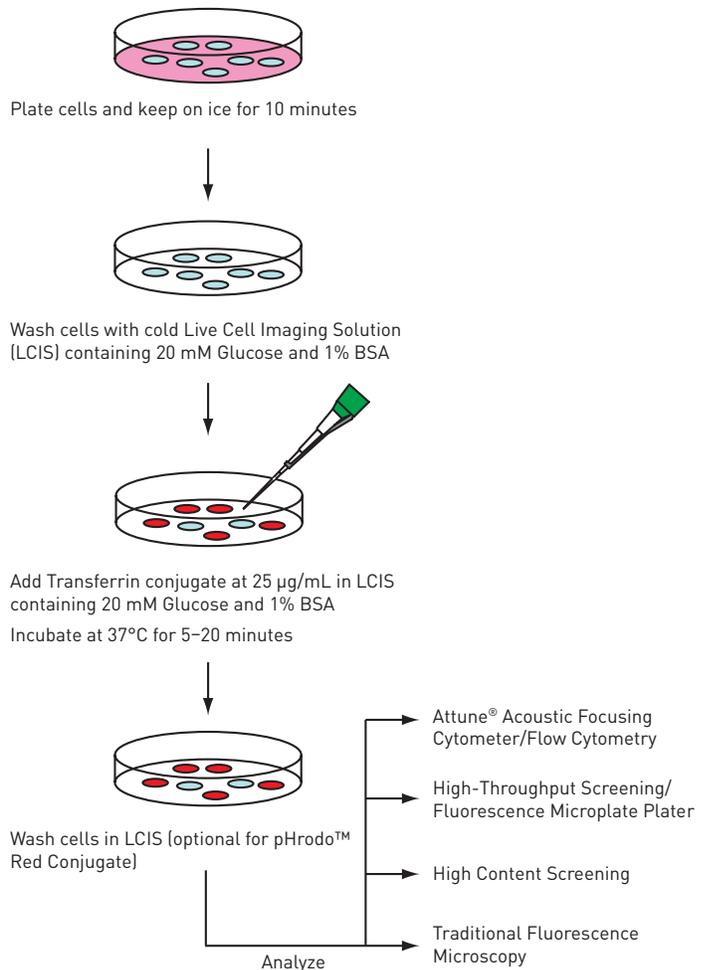


Figure 3 Workflow for fluorescent transferrin conjugates



Guidelines for Use

Wash solution	Although any HEPES-based buffer at pH 7.4 can be used, we recommend using Live Cell Imaging Solution (Cat. no. A14291DJ), supplemented with 20 mM glucose and 1% BSA.
Stock solution	Transferrin conjugates should be reconstituted in deionized water to obtain a 5 mg/mL stock solution in PBS. Store the stock solutions at 2–8°C, protected from light. If the solution needs to be stored for a long time, then 2 mM sodium azide can be added. Do not freeze the solutions.

Application 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 samples. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing non-specific background staining.

Cells can be placed on ice for 10 minutes and cold wash solution can be used to inhibit endocytosis

Make the labeling solution by mixing an aliquot of the stock solution with the wash buffer, and incubate it with the cells at 37°C for 5–30 minutes, and then wash and examine the cells. Washing after labeling is optional for the pHrodo Red™ conjugate. The concentration of the probe in labeling solution needs to be optimized; however, a 25 µg/mL labeling solution in wash solution has been used successfully.

Competition with unlabeled transferrin may be used as a control for non-specific binding of the labeled peptide. We recommend of using 250 µg/mL or ten times as much concentration of unlabeled Transferrin as of labeled Transferrin. This pre-treatment, or co-incubation, will ensure that the Transferrin receptors are internalized, reducing the signal from labeled transferrin.

When using the pHrodo™ Red conjugate, pH can be quantified with the Intracellular pH Calibration Buffer Kit (Cat. no. P35379).

Cells labeled with fluorescent transferrin conjugates may be analyzed by fluorescence microscopy, high content screening (HCS), flow cytometry (Figure 2, page 2), and microplate-based fluorometry or high throughput screening (HTS), using the appropriate filter sets (Figure 3, page 2). Refer to Table 2 (page 2) for peak excitation and emission wavelengths for the fluorescent transferrin conjugates).

Labeling with biotinylated transferrin may be followed by second-step labeling with one of Life Technologies® extensive selection of avidin, streptavidin, or NeutrAvidin® biotin-binding protein conjugates.

Warning

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 it 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.

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. no.	Product Name	Unit Size
P35376	pHrodo™ Red Transferrin Conjugate	1 mg
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
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
Related Products		
P35379	Intracellular pH Calibration Buffer Kit	1 kit
A14291DJ	Live Cell Imaging Solution	500 mL

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