Mammalian Expression Systems

Xfect™ Protein Transfection Reagent

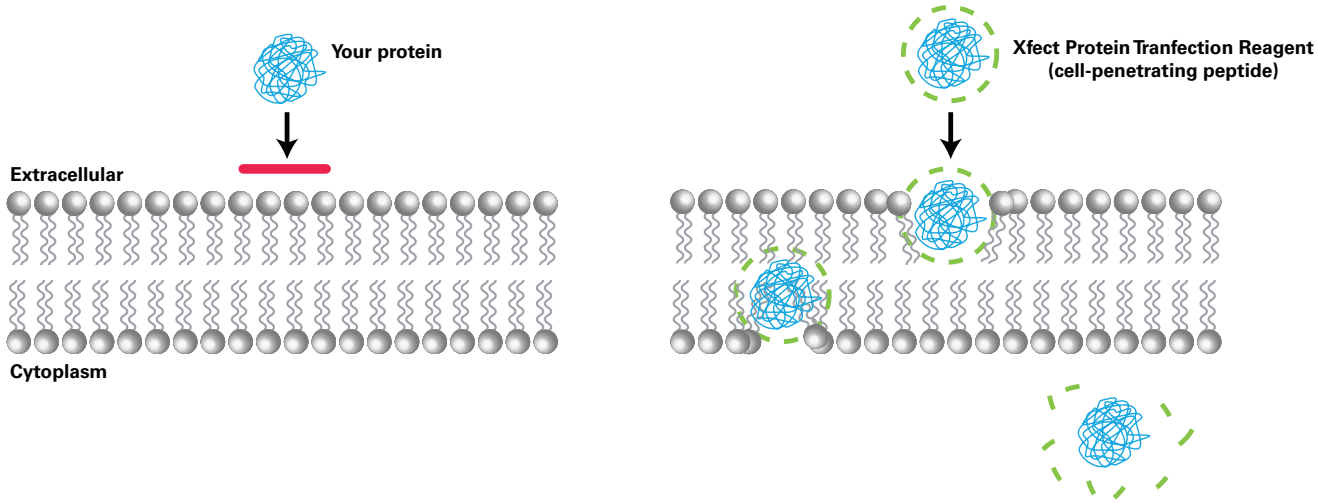
Rapid, high-efficiency, low-toxicity protein transfection

- **Transfect a large amount of active protein**
- **Virtually no cytotoxicity, unlike lipofection**
- **Very high efficiency, even in stem or hematopoietic cells**
- **Simple protocol—assay for your protein in just 2 hours**

Our new **Xfect Protein Transfection Reagent** uses a cell-penetrating peptide developed at Clontech to bind and transport active proteins directly into a wide variety of mammalian cell types, including hard-to-transfect human suspension cell lines and mouse embryonic stem cells.

What is Xfect Protein Transfection Reagent and how does it work?

Xfect Protein Transfection Reagent is a modified peptide with cell-penetrating activity whose amino acid composition enables it to interact with a protein cargo and transport this protein across a cell membrane barrier. Just incubate your protein of interest with Xfect in the supplied buffer for 30 min, then apply the mixture to your cells—and 2 hours later, you are ready to assay for protein activity.



Simple, rapid protein transfection with Xfect Protein Transfection Reagent. Xfect's cell-penetrating activity enables proteins to be transported across membranes of mammalian cells.

Ordering Information		
Product	**Size**	**Cat. No.**
Xfect Protein Transfection Reagent	30 rxns	631323
	100 rxns	631324

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Clontech

Why transfect purified proteins directly?

Protein transfection is extremely rapid (Figure 1) compared to traditional gene expression studies using transfected DNA (1–2 hours compared to 18–48 hours), because it bypasses cellular processes such as transcription and translation. It also facilitates studies involving transient effects of proteins, and avoids potentially harmful, random DNA integration into the genome of the target cells. Xfect Protein Transfection Reagent makes it possible to deliver active proteins (Figures 1 & 2) directly into cells for studies that involve transcriptional regulation, the cell cycle, apoptosis, oncogenesis, epigenetics, cell regeneration, and transdifferentiation.

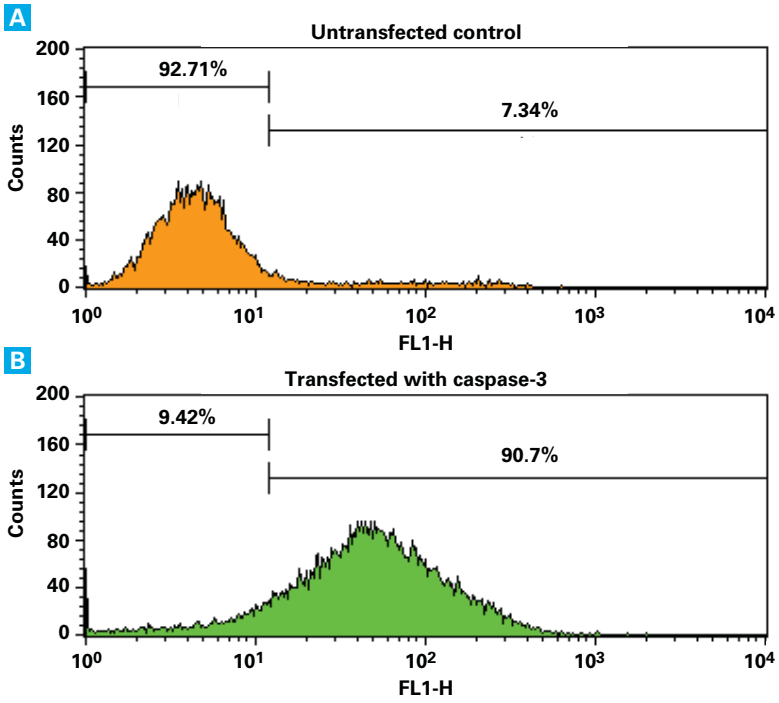

Figure 1. Complete induction of an early apoptosis event within 2 hours via protein transfection of active caspase-3. Apoptosis is commonly detected using annexin V–FITC staining (see Clontech's **ApoAlert™ Annexin V-FITC Apoptosis Kit**, Cat. No. 630109), which detects translocation of phosphatidylserine from the inner (cytoplasmic) leaflet of the plasma membrane to the outer (cell surface) leaflet soon after the induction of apoptosis. Annexin V-FITC staining is detected by flow cytometric measurement of increased fluorescence intensity. We transfected human recombinant caspase-3 using Xfect Protein Transfection Reagent and detected completion of apoptosis after just 2 hr, demonstrating that protein transfection using Xfect is both fast and delivers active protein (**Panel B**). In comparison, other methods for inducing apoptosis require 12 hr for completion of Annexin V staining (1).

What are the advantages of Xfect Transfection Reagent compared to other protein delivery technologies?

The two technologies most frequently used for protein delivery are based on lipids and cell-penetrating peptides. Transfection with lipid-based reagents tends to result in high cytotoxicity, while peptide-based reagents transfect with low efficiency. Xfect Protein Transfection Reagent offers the best advantages of both, i.e., it retains low cytotoxicity (Figures 4 & 5) and delivers more protein to a higher percentage of target cells. (Figures 2, 3 & 6). Moreover, Xfect Protein can transfect cells that are growing at a higher density than competing products, which is important because assays are often performed within a few hours post-transfection and the higher cell densities ensure sufficient material for downstream analysis.

Is the transfected protein active?

Yes, the protein is active. Assays that measure enzyme activity (such as caspase-3 or β-galactosidase) show that these proteins retain their activity after transfection with Xfect Protein Transfection Reagent, while fluorescent proteins retain their ability to fluoresce (Figures 1, 2 & 5).

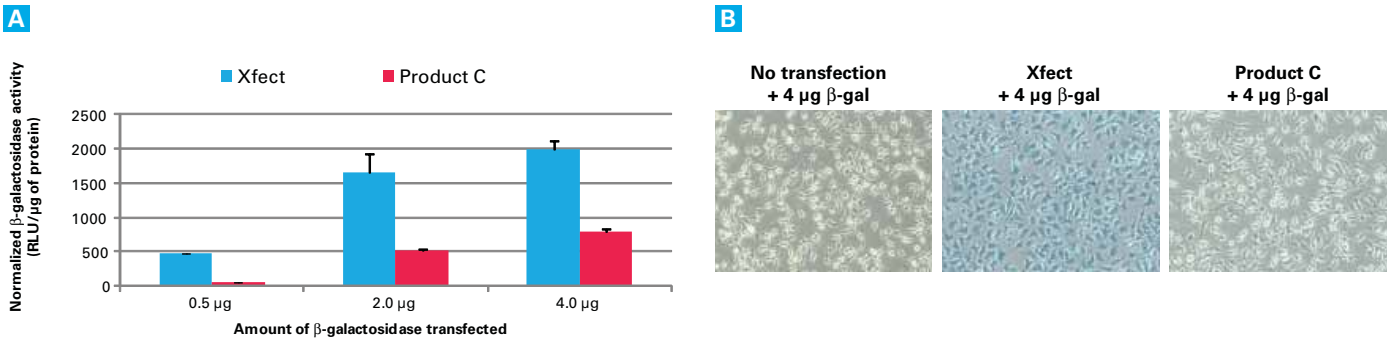

Figure 2. Xfect Protein Transfection Reagent delivers up to 8X more active β-galactosidase into HeLa cells than the leading competitor, Product C. Panel A. Xfect Protein Transfection Reagent or Product C was used to transfect Jurkat cells with various amount of β-galactosidase (β-gal) according to the manufacturers' recommended protocol. One hour post-transfection, the cells were assayed for β-gal activity using Clontech's **Luminescent β-galactosidase Detection Kit II** (Cat. No. 631712). Xfect Protein Transfection Reagent displayed a far higher signal for β-gal than did Product C. **Panel B.** HeLa cells transfected with 4 µg of β-gal demonstrated higher efficiency and a higher amount of β-gal protein per cell when transfection was performed with Xfect Protein Transfection Reagent (middle panel) than with Product C (righthand panel).

Does Xfect Protein Transfection Reagent deliver a high amount of protein?

Yes, Xfect Protein Transfection Reagent delivers far more protein per cell than the leading protein transfection reagent (Product C), a peptide-based reagent, in a broad range of cell types. This was demonstrated when we compared the amounts of β-galactosidase (Figure 2) and fluorescent proteins (Figure 3) delivered by each reagent. When we compared 6 different cell types, Xfect delivered 3–13 fold more protein than Product C (Figure 3). The biggest disadvantage of other protein delivery reagents is their limited ability to monitor a protein's biological effects (due to an inability to deliver sufficient active protein). Xfect Protein delivers more protein per cell than other reagents, so it should expand the applications for protein delivery and/or allow you to use less protein for transfection.

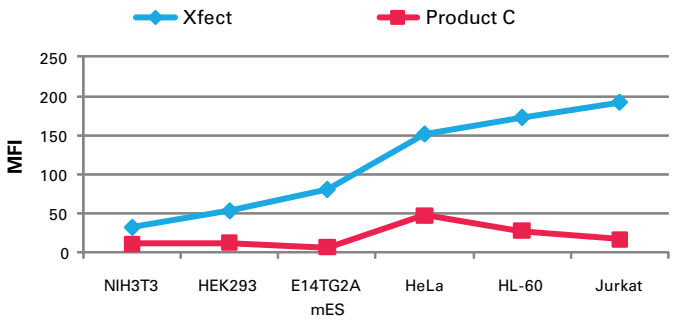

Figure 3. Xfect Protein Transfection Reagent delivers more recombinant fluorescent proteins than Product C into a range of cell types. Xfect Protein Transfection Reagent and Product C were used to transfect a number of cell lines with purified recombinant fluorescent protein (either rAcGFP1 or rDsRed-Express). In all of the cell lines tested, a far greater MFI (mean fluorescence intensity) was detected via flow cytometry after transfection with Xfect Protein Transfection Reagent than after transfection with Product C.

Does Xfect Protein Transfection Reagent affect cell viability?

No, as demonstrated when we performed a WST-1 assay with Clontech's **Premixed WST-1 Cell Proliferation Reagent** (Cat. No. 630118) to compare the effects on HeLa cell proliferation of Xfect Protein Transfection Reagent and Product P (a popular lipid-based protein transfection technology). Xfect did not affect cell proliferation at all compared to untreated cells, while Product P decreased cell proliferation by more than 30% (Figure 4). When we used Xfect to transfect DsRed-Express (a red fluorescent protein) into a commonly used mouse embryonic stem cell line and measured cell viability using trypan blue dye exclusion, the cells retained 100% viability (Figure 5).

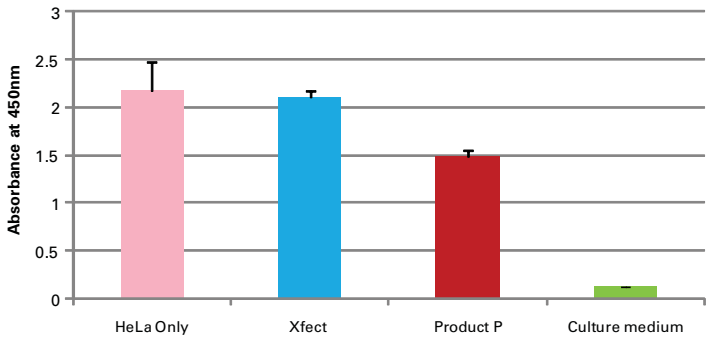

Figure 4. Xfect Protein Transfection Reagent provides much higher cell viability than Product P when transfecting HeLa cells. HeLa cells were seeded in 96-well plate (3 x 10^4 cells per well) one day prior to transfection. The cells were transfected with 0.25 µg of β-galactosidase using Product P or Xfect Protein Transfection Reagent for 4 hours. A WST-1 proliferation assay was performed to assess cytotoxicity in the transfected samples relative to untreated HeLa cells.

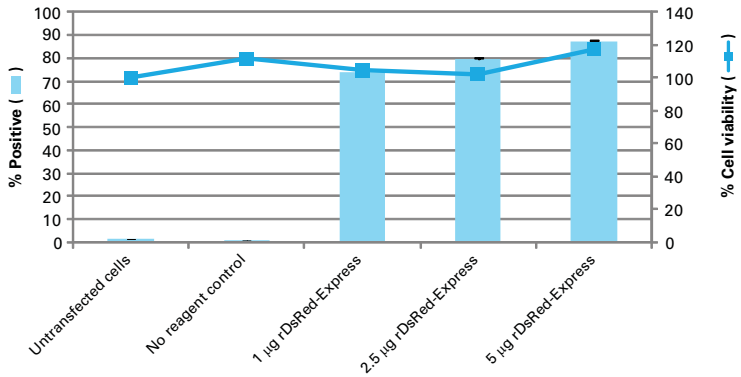

Figure 5. Cytotoxicity is undetectable in mouse embryonic stem cells. 1, 2, and 5 µg of a recombinant red fluorescent protein (rDsRed-Express) were transfected with high efficiency into E14TG2a mES cells. Relative cell viability was measured by a dye exclusion assay and showed that the cells retain 100% viability compared to an untransfected cells control (cells only) and a negative (no reagent) control (5 µg rDsRed-Express added to cells without any transfection reagent).

What is the transfection efficiency of Xfect Protein Transfection Reagent?

The transfection efficiency of Xfect Protein Transfection Reagent can vary depending on the cell type and protein delivered. However, in all cell lines tested, Xfect outperforms Product C. When we transfected AcGFP1 (green fluorescent protein) into several different cell types using Xfect and measured transfection efficiency using flow cytometry, more than 90% of the cells were transfected in 4 out of 8 cell types that we tested and 7 of these 8 showed more than 80% transfection efficiency (Figure 6, Panel A). When immunofluorescence microscopy was used to compare the efficiency of AcGFP1 transfection into HeLa cells using Xfect and Product C (a leading competitor), a high level of transfection was observed only for the cells treated with Xfect (Figure 6, Panel B).

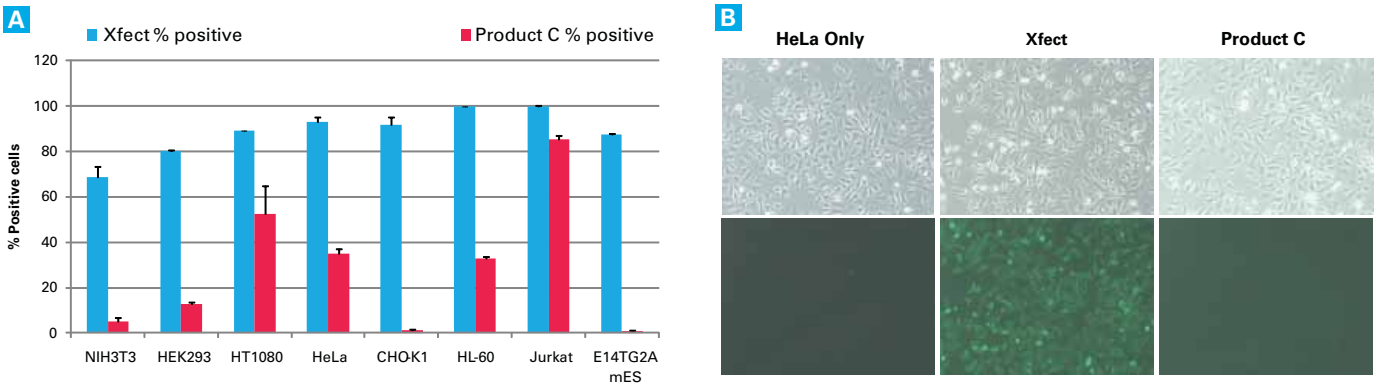

Figure 6. Protein transfection efficiencies across different cell lines: Xfect Protein Transfection Reagent vs. the leading competitor, Product C. Panel A. Xfect Protein Transfection Reagent yields higher transfection efficiencies than Product C across a broad range of mammalian cells, including a number of rodent and human cell lines, hard-to-transfect human suspension cells, and mouse embryonic stem cells. The cells were transfected with 4 µg of recombinant fluorescent protein (either rAcGFP1 or rDsRed-Express) using Xfect Protein Transfection Reagent or Product C according to the manufacturers' recommended protocol. Transfection efficiency was assessed by flow cytometry one hour post-transfection. **Panel B.** HeLa transfected with 5 µg of rAcGFP1 using Product C or Xfect Protein Transfection Reagent.

Will Xfect Protein Transfection Reagent work for all cell types, including cell types that cannot be easily transfected with DNA?

Target cell choices are not limited. We have successfully transfected a variety of mammalian cell types (human and rodent) with high efficiency, including hard-to-transfect human suspension cell lines (Jurkat, HL-60), which were transfected with close to 100% efficiency (Figure 7). Mouse ES cells (E14TG2A) are transfected at an amazing 87% efficiency when using Xfect Protein Transfection Reagent (Figure 6, Panel A), despite the fact that they cannot be transfected using Product C from a leading competitor.

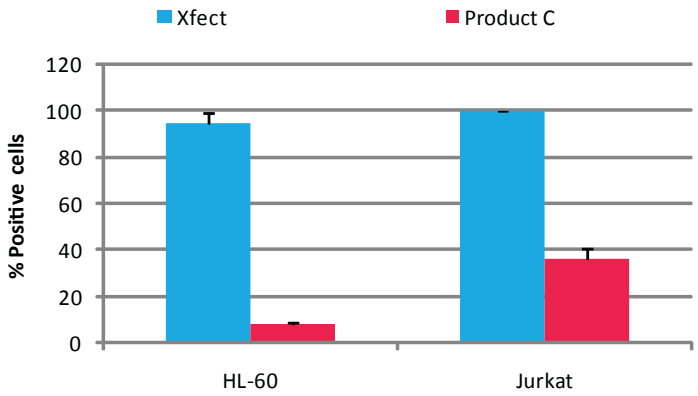

Figure 7. Xfect Transfection Reagent yields extremely high transfection efficiencies even in difficult-to-transfect human suspension cell lines such as HL-60 or Jurkat.

Xfect™ Protein Transfection Reagent...continued

Does Xfect Protein Transfection Reagent work for all proteins?

Since every protein has unique physicochemical characteristics, it is unlikely that any protein transfection reagent will transfect all proteins. However, Xfect Protein Transfection Reagent has been used successfully to transfect numerous proteins with a wide range of molecular weights and physicochemical characteristics. Proteins we have successfully transfected include β-galactosidase, fluorescent proteins such as rAcGFP1 and rDsRed-Express, anti-alpha tubulin antibody, caspase-3, and R-phycoerythrin. R-phycoerythrin is a very large protein (~240 kDa). Optimizing the ratio of reagent to protein will allow you to optimize the conditions for delivery of your protein of interest.

References

1. Martin, S. J. *et al.* (1995) J. Exp. Med. **182**(5):1545–1556.

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