Chariot™

simple, efficient protein delivery

Chariot™ is a revolutionary delivery reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells in less than two hours.

Chariot advantages

- Fast and efficient — up to 95% delivery in under 2 hours
- Non-covalent binding preserves the delivered protein's function
- Works in a variety of cell lines, including hard-to-transfect cells
- Facilitates functional studies in living cells — no fixing required
- Non-cytotoxic, serum independent

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Chariot™ – simple, efficient protein delivery

Chariot™ is a revolutionary delivery reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells in less than two hours[1, 2]. The typical delivery efficiency is 65-95%. After delivery, living cells can be assayed to determine the effects of the introduced materials, without the need for fixing. This makes Chariot ideal for a wide variety of functional studies, including delivery of inhibitory proteins, organelle labeling, screening peptide libraries and transient complementation studies.

Current transfection techniques often use microinjection[3], calcium phosphate coprecipitation[4], cationic liposomes[5], viral vectors[6] and electroporation[7]. While these methods transport DNA into cells, they are often inconvenient and time-consuming. After transfection, the researcher must wait overnight to several days to detect gene expression. A greater drawback is that the cytotoxicity of these methods can make it difficult to determine if any observed changes are caused by the recombinant protein or by the transfection method itself.

The Chariot advantage

Chariot is a peptide that forms a non-covalent complex with the macromolecule to be delivered, which stabilizes the protein, protecting it from degradation during delivery and preserving its function. Upon addition to cells, the complex is rapidly internalized, where it dissociates[1]. The Chariot peptide localizes to the cell nucleus where it is degraded, leaving the macromolecule free to proceed to its target organelle. By directly delivering protein, peptide or antibody, Chariot completely bypasses the transcription-translation process associated with gene expression, saving hours to days of time.

Chariot is non-cytotoxic and efficiently delivers proteins into a wide range of cell lines, including hard-to-transfect PC-12 and primary cells. It has proven to be effective on both adherent and suspension cells. As efficient delivery occurs at 4°C, the Chariot process is independent of the endosomal pathway[1], which can modify macromolecules during internalization.

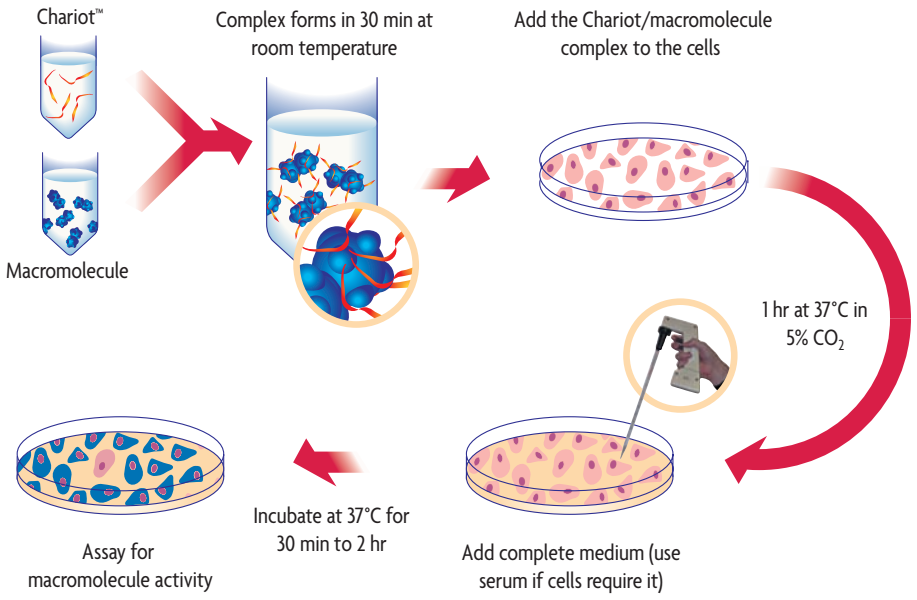

Diagram 1: Flow chart of the Chariot process.
Chariot is very easy to use. Simply combine it with your macromolecule, wait for 30 minutes, then add to your cells. Activity can be assayed in as little as 90 minutes post-delivery.

Proven performance

Chariot's transfection success across multiple cell lines makes it ideal for any cell system. It has been used to efficiently deliver proteins, peptides and antibodies directly into fibroblasts, granulosa cells, protoplasts, endothelial cells, epithelial cells and neuronal cells. Chariot's high delivery efficiency in even difficult-to-transfect cells gives you flexibility in your cell choice. The process is also serum-independent, so delivery can occur in the presence or absence of serum. Chariot enables you, not your delivery reagent, to decide which cell lines are best to use in your experiments.

Specific cell lines that have been successfully transfected using Chariot include:

293	HS-68	HeLa
CEM-SS	COS-7	MDA-MB-231
NIH/3T3	C2C12	Hep G2
Jurkat	PC-12	CV-1
CHO	NCI-H295	SIGC
LLC-PK	NRK	Arabidopsis
Saos-2	IMR90	U87mg
NRK	WI-38	HCA2

Deliver biologically active proteins

The ability of Chariot to deliver biologically active proteins was demonstrated using $p27^{kip1}$, a 27 kDa cyclin-dependent kinase inhibitor that causes cell-cycle arrest in G_1 phase. Flow cytometry (Figures 1A & 1B) and bromodeoxyuridine (BrdU) labeling (Figure 1C) of three different cell lines were performed following delivery of $p27^{kip1}$ by Chariot. HS-68, Jurkat and WI-38 cells were arrested in G_0 phase by serum deprivation for 48 hours, and then released by addition of serum for three hours. 100 µl of 50 nM $p27^{kip1}$ protein was complexed with Chariot for 30 minutes. Chariot alone, $p27^{kip1}$ alone and the Chariot-$p27^{kip1}$ complex were added to the cells. The inability of > 90% of the cells that received the Chariot-$p27^{kip1}$ complex to progress beyond G_1 phase demonstrates efficient delivery of active $p27^{kip1}$.

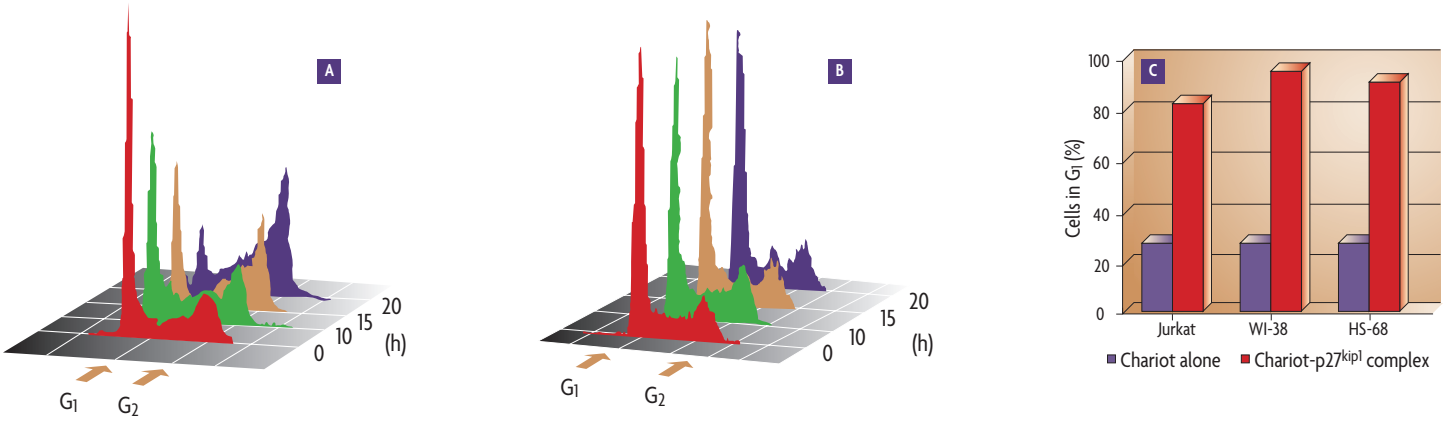

Figure 1: Cell cycle arrest by delivered peptide.
Flow cytometry of HS-68 cells was performed 0, 10, 15 and 20 hours after delivery of the samples. Cells that received Chariot alone (A) and $p27^{kip1}$ alone (data not shown) were able to progress into G_2 phase. Over 90% of the cells that received the Chariot-$p27^{kip1}$ complex (B) remained in G_1 phase. BrdU incorporation experiments were also carried out 14 hours after delivery of the samples. The cells were labeled with BrdU, fixed and then incubated with anti-BrdU antibody and a fluorescent secondary antibody. The percentage of cells in G_1 phase was determined by counting cells on an immunofluorescence microscope (C). Data generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montepellier, France.

Deliver gene silencing reagents

In addition to the original Chariot used to deliver proteins, peptides and antibodies, Active Motif has developed Chariot II, a unique formulation of Chariot that can be used to deliver our exclusive gripNA™ gene silencing reagent. gripNAs are a modified form of negatively charged Peptide Nucleic Acids, which display superior sequence specificity compared to conventional gene silencing reagents. In addition, gripNAs are resistant to nuclease degradation. The ability to deliver gripNAs using Chariot II is a unique advantage as Chariot is non-cytotoxic compared with other lipid-based transfection reagents. And, as Chariot II cannot be used with other nucleic acid based gene silencing tools such as morpholinos or siRNA, gripNAs provide you with a better alternative. Visit the Active Motif gripNA web resource at www.activemotif.com/gripna for all the up-to-date information you need to design, order and use gripNAs.

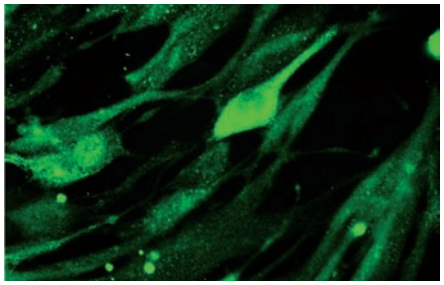

Figure 2: Chariot II delivery of Fluorescein-tagged gripNA.
An 18-mer gripNA probe (1 µM) labeled on its 3´ end with Fluorescein was complexed with Chariot II in PBS and incubated for 30 minutes at 37°C, then overlaid onto cultured HS-68 cells for 1 hour. Cells were washed extensively prior to observation. Data provided by Dr. L. Chaloin, Dr. M. Morris and Dr. G. Divita, CNRS, Montpellier, France.

Deliver antibodies directly into live cells

Chariot successfully delivers a variety of antibodies directly into live mammalian cells using our quick and simple protocol. The ability of Chariot to deliver antibodies into cells was demonstrated using FITC-labeled anti-Actin and anti-Lamp 1 antibodies. The labeled antibodies were targeted to actin filaments (Figure 3) as well as to endosomes (Figure 4), and observed after delivery by fluorescence microscopy.

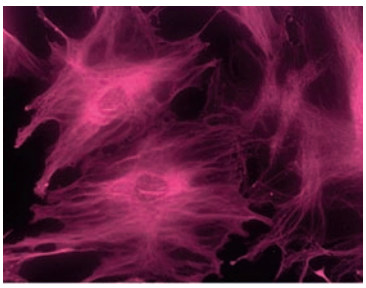

Figure 3: Delivery of fluorescent Actin antibody. Actin filament labeling of human fibroblast (HS-68) cells using a 1/1000 dilution of an anti-Actin antibody. Unfixed cells were observed 2 hours post-delivery.

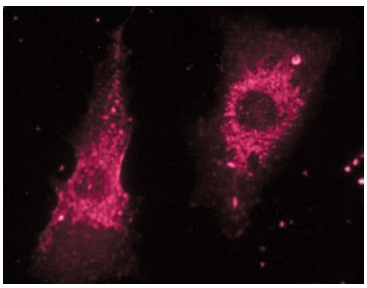

Figure 4: Delivery of fluorescent Lamp-1 antibody. Endosome labeling of HS-68 cells using a 1/500 dilution of anti-Lamp 1 antibody. Unfixed cells were observed 2 hours post-delivery.

Targeted delivery

An advantage of the non-covalent Chariot-macromolecule complex is that, after delivery, the macromolecule is biologically active and free to proceed to its target organelle, providing it contains specific signaling sequences. This unique property was demonstrated using a 51-mer nuclear protein (Figure 5) and a 32-mer cytoplasmic peptide (Figure 6).

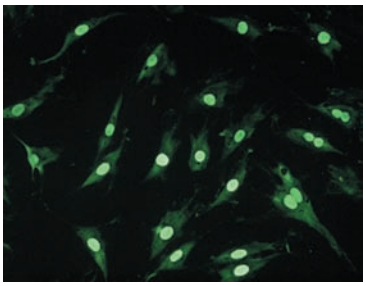

Figure 5: Targeted protein delivery. Chariot was complexed with a fluorescently labeled 51-mer nuclear protein at a 20:1 ratio and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

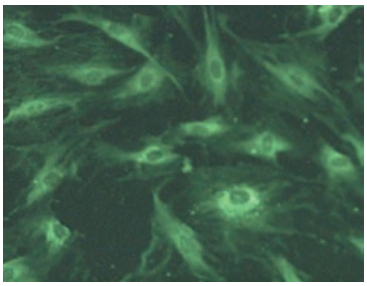

Figure 6: Chariot delivery of peptide. Chariot was complexed with a fluorescently labeled 32-mer cytoplasmic peptide at a 20:1 ratio and delivered into human fibroblasts. Unfixed cells were observed 90 minutes post-delivery.

Get Chariot and get results

Chariot speeds and simplifies a variety of functional studies because it efficiently delivers biologically active proteins, peptides and antibodies directly into live mammalian cells. This means you'll get conclusive data immediately, rather than having to wait days for ambiguous expression data. To find more information about Chariot, please visit our website at www.activemotif.com.

Ordering information

Product	Format	Catalog No.
Chariot™	25 rxns	30025
	100 rxns	30100
β-Galactosidase Staining Kit	75 rxns	35001

CONTENTS & STORAGE

Sufficient reagents are included for either 25 or 100 reactions, with each reaction being the equivalent of a 35 mm plate or a single well of a six-well plate. Chariot Kits contain Chariot Protein Delivery Reagent, β-galactosidase positive control, PBS and sterile H_2O. β-Galactosidase Staining Kits contain Glutaraldehyde fixative, X-gal, $MgCl_2$, potassium ferrocyanide and potassium ferricyanide. Sufficient reagents are included to perform 75 assays in 35 mm dishes. Store all components at -20°C. All reagents are guaranteed stable for 6 months when stored properly.

REFERENCES

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