Chariot™

Simple, efficient protein delivery

(version 01/02)

Catalog Nos. 30025 & 30100

Active Motif North America
5431-C Avenida Encinas
Carlsbad, California 92008, USA
Toll free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351

Active Motif Europe
104 Avenue Franklin Roosevelt
B-1330 Rixensart, Belgium
Toll free: +800 222 95 430
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050

Funakoshi Japan
Telephone: +81 3 5684 1620
Fax: +81 3 5684 1775

TABLE OF CONTENTS **Page**

Overview

Description

Chariot™ is a proprietary transfection reagent suitable for the delivery of proteins, peptides and antibodies into a variety of cultured mammalian cells. Chariot Kits come in two sizes, with sufficient Chariot Transfection Reagent for either 25 or 100 reactions in 6-well or 35 mm plates when using the β-galactosidase control.

Plate size	Chariot (µl)	No. of Transfections per Kit (25-reaction Kit)	No. of Transfections per Kit (100-reaction Kit)
24-well	2	75	300
12-well	3.5	42	171
6-well or 35 mm	**6**	**25**	**100**
60 mm	20	7	30
100 mm	50	3	12

Kit Contents

Component	Quantity		Composition	Storage
	25 rxns	100 rxns		
Chariot Transfection Reagent	0.3 mg	1.2 mg		-20°C for up to 6 months
PBS	1 ml	1 ml	1.5 mM KH_2PO_4 150 mM $NaCl_2$ 5 mM Na_2HPO_4	-20°C
β-galactosidase (Positive Control)	25 µg	25 µg		-20°C for up to 6 months
Sterile H_2O	1 ml	1 ml		-20°C

Quality Control

- Chariot Transfection Reagent is functionally tested by delivery of protein and antibody into three different cell lines (HS-68, NIH 3T3 and HeLa)
- Chariot Transfection Reagent is tested for the absence of bacterial and fungal contamination in DMEM cell culture media supplemented with 10% FBS
- Chariot Transfection Reagent is non-cytotoxic at the recommended concentrations

Introduction

Chariot™ is a revolutionary new transfection reagent capable of efficiently delivering proteins, peptides and antibodies into cultured mammalian cells in less than two hours.

Current transfection techniques include microinjection[1], calcium phosphate coprecipitation[2], cationic liposomes[3], viral vectors[4] and electroporation[5]. These methods are capable of transporting DNA into cells, but the techniques can be cumbersome and even cytotoxic. Once transfection has been completed, the researcher must wait 12-80 hours post-transfection to detect expression of the gene of interest.

Recently, novel methods to deliver proteins have been reported and commercialized, *i.e.* HIV-1 TAT, *Drosophila* Antennapedia homeotic transcription factor and herpes simplex virus-1 DNA binding protein VP22 (for a recent review, see ref. 6). Transduction via TAT-fusion proteins results in inactivation and denaturation of the protein. To deliver an active protein, correct renaturation is required upon internalization. In addition, TAT must be covalently linked to the compound or macromolecule to be delivered by a chemical reaction.

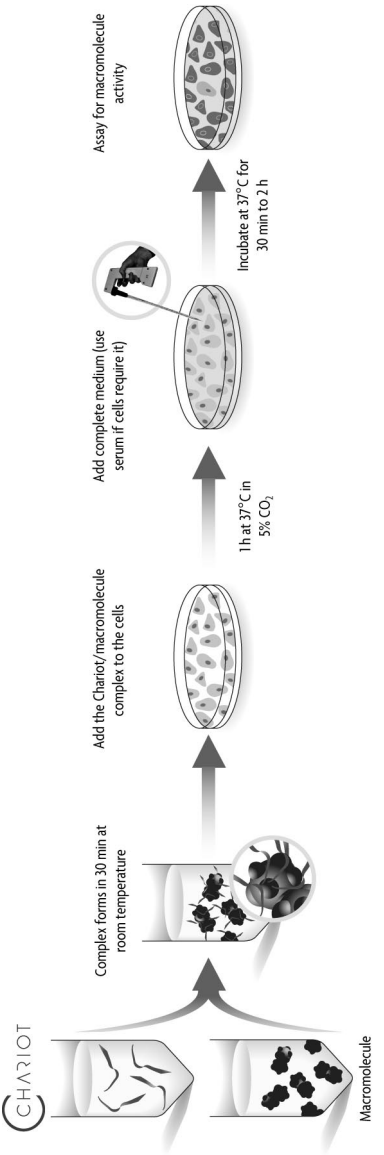
Penetratin 1 is a 16 amino acid peptide corresponding to the third helix of the homeodomain of Antennapedia protein. Activated Penetratin has an N-terminal pyridyl disulfide that is used to covalently couple it to the macromolecule to be delivered. However, chemical coupling can be cumbersome and requires the macromolecule being delivered to carry a free thiol group.

Another delivery system utilizes the translocation properties of the 38 kDa herpes simplex virus-1 DNA binding protein VP22. VP22 must be fused to the peptide/protein to be delivered and, therefore, requires the construction of a suitable expression vector.

Unlike these current transfection techniques, Chariot forms a non-covalent complex with the protein, peptide or antibody of interest. This completely bypasses the transcription-translation process associated with gene expression, reducing the time until the cells can be assayed from days to under two hours. The Chariot-macromolecule complex stabilizes the macromolecule and helps to protect it from degradation during the transfection process[7, 8]. Upon internalization, the complex dissociates and the macromolecule is free to proceed to its target organelle. Moreover, efficient delivery is observed at 4°C, suggesting that the delivery mechanism is independent of the endosomal pathway[8]. Therefore, the macromolecule is not subjected to the conditions of that pathway, which can modify the structure of the macromolecule during internalization. Chariot is non-cytotoxic and serum-independent[8]. It is ideal for *in vivo* studies because fixing is not required. Chariot has been used to efficiently transfect a variety of cell lines, including:

293	**HS-68**	**HeLa**	**CEM-SS**
COS-7	**NIH 3T3**	**C2C12**	**HepG2**
Jurkat	**PC-12**		

Flow Chart of Process



Protocols

Preparation of Chariot

Chariot is provided as a lyophilized powder. Store at -20°C.

25-reaction Kit: Resuspend the entire contents of the Chariot vial in 150 µl sterile H_2O. Mix gently by tapping tube.

100-reaction Kit: Resuspend the entire contents of the Chariot vial in 600 µl sterile H_2O. Mix gently by tapping tube.

After resuspension, store at -20°C. Because it is best to avoid repeated freeze/thaw cycles, it is recommended to aliquot the Chariot into tubes containing the amount you expect to use in a typical experiment prior to freezing.

Preparation of β-galactosidase

The β-galactosidase positive control protein is provided as a lyophilized powder. Store at -20°C. 25 µg of protein is provided in both the 25- and 100-reaction kits. Resuspend the entire contents of the β-galactosidase positive control vial in 100 µl sterile H_2O. This makes a 0.25 µg/µl stock. Use 0.5-1.0 µg β-galactosidase per transfection reaction. After resuspension, store at -20°C. Again, aliquot into several tubes to avoid repeated freeze/thaw cycles.

Transfection Protocol for 6-well or 35 mm plates

These conditions are recommended as guidelines only. Efficient transfection may require optimization of reagent concentration, cell number and exposure time of cells to the Chariot-macromolecule complex. Conditions should also be optimized for each cell line and kept consistent to obtain reproducible results. This procedure has been optimized for the transfection of adherent cells using the β-galactosidase positive control.

1. In a 6-well or a 35 mm tissue culture plate, seed 0.3 x 10^6 cells per well in 3 ml of complete growth medium.

   Note: Adjust the number of cells and volumes accordingly if using cells or culture plates of different sizes (See Table 1). These numbers are used with HeLa cells.

Table 1

Plate	Surface Area (mm^2)	Cells	Growth Medium (ml)
24-well	200	0.05 x 10^6	0.5-1.0
12-well	401	0.1 x 10^6	1-2
6-well or 35 mm	**962**	**0.3 x 10^6**	**3-5**
60 mm	2827	0.8 x 10^6	5
100 mm	7854	2.2 x 10^6	10

2. Incubate the cells at 37°C in a humidified atmosphere containing 5% CO_2 until the cells are 40-50% confluent.

   Note: The transfection efficiency may be sensitive to culture confluency, so it may be necessary to optimize cell density for each cell line.

3. After resuspension, Chariot is ready to use for protein and antibody transfections. For peptide or low molecular weight protein (< 10 kDa) transfections, the Chariot solution must be diluted 1:10 in sterile H_2O.

4. For 6-well or 35 mm culture plates, dilute the amount of your macromolecule listed below into 100 µl of PBS for each transfection reaction.

   Note: If using culture plates of different sizes, use the amount of protein, peptide or dilution of antibody listed below and dilute into the final volume of PBS listed in Table 2.

Table 2

Plate	Final volume of macromolecule dilution in PBS (µl)
24-well	50
12-well	100
6-well or 35 mm	**100**
60 mm	200
100 mm	200

Protein: Use 0.5-1 µg of protein per transfection reaction.

Peptide: Use 100-500 ng of peptide or low molecular weight protein per transfection reaction.

Antibody: Make a 1/500, 1/1000 or 1/2500 dilution of your antibody in 100 µl of PBS per reaction. Calculate your dilution using the appropriate Final Transfection Volume listed in Table 3. **For example**, if you have a 3 µg/µl stock of antibody and would like to make a 1/500 dilution for use in a 35 mm plate, add 3.6 µg (1.2 µl) of antibody to a final volume of 100 µl of PBS.

3 µg/µl / 500 (dilution) x 600 µl (Final Transfection Volume) = 3.6 µg antibody

3.6 µg / 3 µg/µl = 1.2 µl of stock solution. Add to 98.8 µl PBS.

Table 3

Plate	Final Transfection Volume (µl)
24-well	200
12-well	350
6-well or 35 mm	**600**
60 mm	2000
100 mm	5000

5. In a separate tube, dilute the appropriate volume of Chariot into 100 µl sterile water (See Table 4).

Note: Diluting Chariot into DMSO has been shown to improve delivery of some antibodies. It has also provided more uniform delivery of some proteins, including the β-galactosidase control. Please refer to the sections in the Troubleshooting Guide that discuss the use of DMSO.

Table 4

		Protein/Antibody*	Peptide/LMW Proteins**
Plate	Sterile H_20 for Chariot dilution (µl)	Volume of Chariot (µl)	Volume of a 1/10 dilution of Chariot (µl)
24-well	50	2	2
12-well	100	3.5	3.5
6-well or 35 mm	**100**	**6**	**6**
60 mm	200	20	20
100 mm	200	50	50

- For multiple transfections, do not use a master mix that exceeds the volume required for 4 transfections per tube, as this may cause aggregation.

* Detection of fluorescently labeled antibodies in cells requires a large amount of antibody to be delivered. Depending on the sensitivity required to detect your antibody, it may be necessary to increase the amount of Chariot and antibody used. However, excessive Chariot may result in aggregate formation.

** Chariot interacts via hydrophobic interactions. Each peptide or protein will have a different hydrophobicity. This is more apparent with small, lower molecular weight molecules. Therefore, the amount of Chariot may need to be titrated to determine the optimal amount.

6. Add the 100 µl macromolecule dilution to the 100 µl Chariot dilution. (It is necessary to make the Chariot-macromolecule complex in a concentrated solution. This concentrated solution will be added to the cells and then be diluted to the Final Transfection Volume.)
7. Incubate at room temperature for 30 minutes to allow the Chariot-macromolecule complex to form.
8. Aspirate the medium from the cells to be transfected.
9. Wash the cells with PBS (Optional).
10. Overlay the cells with the 200 µl Chariot-macromolecule complex. Add 400 µl serum-free medium to the overlay to achieve the Final Transfection Volume of 600 µl for a 6-well or 35 mm plate (See Table 5).

Table 5

Plate	Volume of Chariot-Macromolecule Complex (µl)	Serum-Free Medium (µl)	Final Transfection Volume (µl)
24-well	100	100	200
12-well	200	150	350
6-well or 35 mm	**200**	**400**	**600**
60 mm	400	1600	2000
100 mm	400	4600	5000

11. Incubate at 37°C in a humidified atmosphere containing 5% CO_2 for one hour.
12. Add 1 ml of complete growth medium to the cells. **Do not** remove the Chariot-macromolecule complex. Continue to incubate at 37°C in a humidified atmosphere containing 5% CO_2 for 30 minutes to 2 hours.

    Peptide: 0.5-1 hour

    Protein: 1-2 hours

    Antibody: 2 hours

13. Process the cells for observation or detection assays. Cells may be fixed or observed directly.

Transfection Protocol for 96-well Plates or 8-well Chamber Slides

These conditions are recommended as guidelines only. Efficient transfection may require optimization of reagent concentration, cell number and exposure time of cells to the Chariot-macromolecule complex. Conditions should also be optimized for each cell line and kept consistent to obtain reproducible results. This procedure has been optimized for the transfection of adherent cells using the β-galactosidase positive control.

1. In a 96-well plate or an 8-well Chamber Slide, seed 1.0 x 10^4 cells per well in 200 µl of complete growth medium.
2. Incubate the cells at 37°C in a humidified atmosphere containing 5% CO_2 until the cells are 40-50% confluent.

   Note: The transfection efficiency may be sensitive to culture confluency, so it may be necessary to optimize cell density for each cell type.

3. After resuspension, Chariot is ready to use for protein and antibody transfections. For peptide or low molecular weight protein (< 10 kDa) transfections, the Chariot solution must be diluted 1:10 in sterile H_2O.
4. For a 96-well plate or an 8-well Chamber Slide, dilute the amount of your macromolecule listed below into 10 µl of PBS for each transfection reaction.

   Protein: Use 0.2-1 µg of protein per transfection reaction.

   Peptide: Use 50-500 ng of peptide or low molecular weight protein per transfection reaction.

   Antibody: Make a 1/500, 1/1000 or 1/2500 dilution of your antibody in 10 µl of PBS per reaction. Calculate your dilution using the Final Transfection Volume of 100 µl per well. (See antibody dilution example on page 5.)

5. In a separate tube, dilute 0.5-1 µl of Chariot for each protein or antibody* transfection (or 0.5-1 µl of a 1:10 dilution of Chariot for peptide/LMW protein** transfections) into 10 µl of sterile H_2O per transfection.

   Note: Diluting Chariot into DMSO has been shown to improve delivery of some antibodies. It has also provided more uniform delivery of some proteins, including the β-galactosidase control. Please refer to the sections in the Troubleshooting Guide that discuss the use of DMSO.

   • For multiple transfections, do not use a master mix that exceeds the volume required for 4 transfections per tube, as this may cause aggregation.

   * Detection of fluorescently labeled antibodies in cells requires a large amount of antibody to be delivered. Depending on the sensitivity required to detect your antibody, it may be necessary to increase the amount of Chariot and antibody used. However, excessive Chariot may result in aggregate formation.

** Chariot interacts via hydrophobic interactions. Each peptide or protein will have a different hydrophobicity. This is more apparent with small, lower molecular weight molecules. Therefore, the amount of Chariot may need to be titrated to determine the optimal amount.

6. Add the 10 µl macromolecule dilution to the 10 µl Chariot dilution. (It is necessary to make the Chariot-macromolecule complex in a concentrated solution. This concentrated solution will be added to the cells and then be diluted to the Final Transfection Volume.)
7. Incubate at room temperature for 30 minutes to allow the Chariot-macromolecule complex to form.
8. Aspirate the medium from the cells to be transfected.
9. Wash the cells with PBS (Optional).
10. Overlay the cells with the 20 µl Chariot-macromolecule complex. Add 80 µl serum-free medium to the overlay to achieve the Final Transfection Volume of 100 µl for each well.
11. Incubate at 37°C in a humidified atmosphere containing 5% CO_2 for one hour.
12. Add 100-200 µl of complete growth medium to the cells. **Do not** remove the Chariot-macromolecule complex. Continue to incubate at 37°C in a humidified atmosphere containing 5% CO_2 for 30 minutes to 2 hours.

    Peptide: 0.5-1 hour

    Protein: 1-2 hours

    Antibody: 2 hours

13. Process the cells for observation or detection assay. Cells may be fixed or observed directly.

Transfection Protocol for Suspension Cells

These conditions are recommended as guidelines only. Efficient transfection may require optimization of reagent concentration, cell number and exposure time of cells to the Chariot-macromolecule complex. Conditions should also be optimized for each cell line and kept consistent to obtain reproducible results.

1. Use Table 1 on page 4 as a guideline for the number of cells needed per transfection. The numbers of cells recommended for seeding of adherent cells are also recommended for suspension cells.
2. The Chariot-macromolecule complex is assembled in the same manner as described for adherent cells. See Steps 3-7 on pages 5-7.
3. Collect the suspension cells by centrifugation at 200-400 x *g* for 5 minutes. Remove the supernatant.
4. Wash the cells twice with 1X PBS.
5. Centrifuge at 200-400 x *g* for 5 minutes to pellet the cells. Remove the supernatant.
6. Resuspend the cell pellet in the Chariot-macromolecule complex. Add serum-free medium to achieve the Final Transfection Volume.
7. Incubate at 37°C in a humidified atmosphere containing 5% CO_2 for one hour.
8. Add complete growth medium to the cells. **Do not** remove the Chariot-macromolecule complex. Continue to incubate at 37°C in a humidified atmosphere containing 5% CO_2 for 30 minutes to 2 hours.

   Peptide: 0.5-1 hour

   Protein: 1-2 hours

   Antibody: 2 hours
9. Process the cells for observation or detection assays. Cells may be fixed or observed directly.

β-galactosidase Positive Control

β-galactosidase is an enzyme that hydrolyses β-galactosides, such as lactose and the artificial chromogen X-gal. β-galactosidase is composed of four identical subunits. When β-galactosidase hydrolyses X-gal, it produces a blue color that can be visualized under a bright field microscope. A 119 kDa subunit of the β-galactosidase protein is provided in this kit as a positive control for the Chariot Transfection Reagent. See page 4 for instructions on resuspension of the β-galactosidase positive control. After resuspension, the β-galactosidase control can be transfected into cultured mammalian cells using the protein transfection procedure. Our best results with the β-galactosidase control occur when using Chariot that has been diluted into 60% DMSO. Please refer to the sections in the Troubleshooting Guide that discuss the use of DMSO. After transfection, the cells may be stained to assay for the efficiency of the transfection. For your convenience, Active Motif offers a β-galactosidase Staining Kit (Catalog No. 35001) for this purpose.

β-galactosidase Staining Protocol

Make stock solutions:

400 mM potassium ferricyanide	Store at -20°C
400 mM potassium ferrocyanide	Store at -20°C
200 mM magnesium chloride	Store at -20°C
20 mg/ml X-gal 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside resuspended in DMF (N, N-Dimethyl-formamide)	Store at -20°C in the dark
10X PBS	Store at room temperature
10X Fixing Solution 20% formaldehyde 2% glutaraldehyde in 10X PBS	Store at -20°C

Staining Protocol

Note: Use volumes appropriate for the size of the plate or dish. Ensure that the Fixing and Staining Solutions cover the cells.

1. Remove the growth medium from the transfected cells.
2. Rinse the cells 3 times with 1X PBS.
3. Add 1X Fixing Solution. (Dilute the 10X stock in sterile water to make a 1X solution.) Incubate at room temperature for 5-10 minutes.
4. Prepare the Staining Solution. **Staining Solution should be made fresh each time.**

250 µl 400 mM potassium ferricyanide	(4 mM final)
250 µl 400 mM potassium ferrocyanide	(4 mM final)
250 µl 200 mM magnesium chloride	(2 mM final)
1.25 ml X-gal (20 mg/ml in DMF)	(1 mg/ml final)
23 ml 1X PBS	
25 ml total	

5. Rinse the cells twice with 1X PBS.
6. Add the Staining Solution to the cells.
7. Incubate the cells at 37°C for 30 minutes to 2 hours.
8. Check the cells under a microscope.
9. Calculate the percent of cells transfected with β-galactosidase.

$$\frac{\text{Total No. of blue cells}}{\text{Total No. of cells}} \times 100 = \%\ \text{transfection}$$

References

1. Capecchi, M.R. (1980) *Cell* 22: 479-488.
2. Graham, F.L. and van der Eb, A.J. (1973) *Virology* 52: 456-467.
3. Felgner, P.L. *et al.* (1987) *Proc. Natl. Acad. Sci.* USA 84: 7413-7417.
4. Cepko, C.L. *et al.* (1984) *Cell* 37: 1053-1062.
5. Neumann, E. *et al.* (1982) *EMBO J.* 7: 841-845.
6. Schwarze, S.L. *et al.* (2000) *Trends Cell Biol.* 10: 290-295.
7. Morris, M. (1999) *et al. J. Biol. Chem.* 274 (35): 24941-24946.
8. Morris, M. *et al.* (2001) *Nature Biotech.* 19: 1173-1176.

Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
Low Transfection Efficiency	Cell density	Use adherent cells at a confluency of 40-50%.
	Chariot or macromolecule dilutions were performed in larger volumes than recommended	Dilute the macromolecule into the final volume of PBS recommended in Table 2. Dilute Chariot in the volume of sterile water recommended in Table 4. It is necessary to make the Chariot macromolecule complex in a concentrated solution. For multiple transfections, do not use a master mix that exceeds the volume required for 4 transfections per tube, as this may cause aggregation.
	Chariot and macromolecule were not complexed for 30 minutes	Combine the Chariot dilution and the macromolecule dilution together and allow the complex to form at room temperature for 30 minutes.
	Improper storage of Chariot	Chariot may form aggregates over time if not stored properly. You may need to sonicate the Chariot dilution just before complex formation. We recommend placing the Chariot dilution in a water bath and sonicating the water at close proximity with an ultrasonic processor. At Active Motif, we routinely use Sonics Vibra-cell™ for 1 minute at an amplitude of 30 and a 1-second pulse.
	Medium that contained serum was used to dilute the Chariot-macromolecule complex	Repeat the transfection using serum-free medium to dilute the Chariot-macromolecule complex to the Final Transfection Volume.
	Exposure of Chariot-macromolecule complex to cells was insufficient	Leave the Chariot-macromolecule complex on the cells for a minimum of two hours.
	Using a cell line other than those tested with Chariot	Try the transfection in a cell line proven to work with Chariot. Chariot has been shown to work in the cell lines stated in this manual for protein, peptide or antibodies.
	A suboptimal amount of macromolecule was used for the transfection	You may need to titrate the amount of macromolecule (protein, peptide or antibody) used in the transfection.
	A suboptimal amount of Chariot was used for the transfection	You may need to titrate the amount of Chariot used in the transfection. Chariot interacts via hydrophobic interactions. Each peptide or protein will have a different hydrophobicity and may require a different amount of Chariot. Depending on the sensitivity required for antibody detection it may be necessary to alter the amount of Chariot used. Detection of fluorescently labeled antibodies in cells requires a large amount of antibody to be delivered. This may mean increasing the amount of Chariot used.

Troubleshooting Guide (cont.)

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
Low Transfection Efficiency (cont.)	Diluting Chariot into DMSO may improve antibody delivery	Diluting Chariot into DMSO has improved the delivery of some, but not all, antibodies. If delivery of your antibody is low, try diluting Chariot into 60-80% DMSO instead of water. After the Chariot dilution is combined with the antibody dilution, the complex mixture will have a final concentration of 30-40% DMSO. This may need to be lowered for certain cell lines, as this amount of DMSO may be toxic. However, a final concentration of complex mixture below 10-20% DMSO has not been shown to improve antibody delivery.
Protein delivery is localized to a few regions of the cell	Diluting Chariot into DMSO may make protein delivery more uniform	With some proteins that we have tested, delivery of the protein seems to be localized to specific regions in the cell. Diluting Chariot into DMSO instead of water has made the delivery of some of these proteins more uniform. Dilute the Chariot into 40-60% DMSO. After the Chariot dilution is combined with the protein dilution, the complex mixture will have a final concentration of 20-30% DMSO. However, this amount of DMSO may be toxic to some cell lines.
Aggregate Formation	Improper storage of Chariot	Chariot may form aggregates over time if not stored properly. You may need to sonicate the Chariot dilution just before complex formation. We recommend placing the Chariot dilution in a water bath and sonicating the waterbath with an ultrasonic processor, such as a Sonics Vibra-cell™, for 1 minute at an amplitude of 30 and a 1-second pulse.
	Excess Chariot used	Decrease the amount of Chariot used in the transfection.
Signs of cytotoxicity. **Note:** Chariot is non-cytotoxic to cells at the recommended concentrations.	Transfected macromolecule may be cytotoxic	Transfect the macromolecule at a lower concentration. Compare cells only, cells with Chariot alone and cells with macromolecule alone. Chariot should not affect cell viability. Transfect the β-galactosidase control protein.
	Culture may be contaminated with mycoplasma	Treat the cells to eliminate mycoplasma.
	Cells may not be healthy	Check the incubator (CO_2 and temperature levels). Check the media.
	Too much DMSO present	Reduce the final concentration of DMSO so that the complex mixture will have a final concentration below 40%. Antibody delivery will occur even in the absence of DMSO.

Technical Services

If you need assistance at any time, please call an Active Motif Technical Service Department at one of the numbers listed below.

Active Motif North America
5431-C Avenida Encinas
Carlsbad, CA 92008
USA
Toll Free Tel: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe
104 Avenue Franklin Roosevelt
B-1330 Rixensart
Belgium
Toll Free Tel: +800 222 95 430
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Funakoshi Japan
Telephone: +81 3 5684 1620
Fax: +81 3 5684 1775
E-mail: reagent@funakoshi.co.jp

Visit Active Motif on the worldwide web at **http://www.activemotif.com**

At this site:

- Read about who we are, where we are, and what we do
- Review data supporting our products and the latest updates
- Enter your name into our mailing list to receive our catalog, *Motifvations* newsletter and notification of our upcoming products
- Share your ideas and results with us
- View our job opportunities

Don't forget to bookmark our site for easy reference!