DeliverX™ Peptide Transfection Kit

User Manual

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Panomics

Panomics, Inc.

DeliverX Peptide Transfection Kit User Manual

Citing DeliverX in Publications

When describing a procedure for publication using this product, we would appreciate it if you would refer to it as the DeliverX™ Peptide Transfection Kit.

If a paper cites a DeliverX product and is published in a research journal, the lead author(s) may receive a travel stipend for use at a technology conference or tradeshow by sending a copy of the paper to our technical support group at techsupport@panomics.com or via fax at (510) 818-2610.

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Contents

DeliverX Peptide Transfection Kit and Manual Overview

About the Kits

Our DeliverX Peptide Transfection Kit is suitable for transfection of most cell types. DeliverX Peptide Transfection Kits contain the reagents required to efficiently transfect peptides into most cell types with minimal cell damage and good reproducibility when following the optimization guidelines provided in this User Manual.

The DeliverX Peptide Transfection Kit is designed to efficiently deliver a wide range of hydrophobic and polar peptides that interfere with the function of targeted cellular proteins. We have successfully transfected 40+ FITC-labeled peptides into the following cell lines with no visible effects on cellular morphology and with cell viabilities over 70%:

Cell Line	% Efficiency of Transfection
NIH3T3	> 70
HeLa	> 70
3T3L1	> 70
C2C12	> 70
Differentiated C2C12	> 30
Human astrocytes, CCF-STTG1	> 70
MEF	> 70

DeliverX Transfection Mechanism

DeliverX transfection reagents are based on the novel delivery technology called “MPG”. This technology was developed at Centre de Recherches en Biochimie Macromoléculaire (CNRS) in Montpelier, France in the laboratory of Dr. F. Heitz and Dr. G. Divita.

MPG technology uses virus-derived amphipathic peptides that directly interact with peptide cargos to form non-covalent nanoparticles (150-200 nm) capable of diffusing through the plasma membrane and releasing their contents inside the cell. The mechanism of entry is receptor-independent, involves MPG/lipid interactions, and avoids the endocytic pathway, thereby preventing endosomal or lysosomal degradation of cargos.

About the Manual

The manual contains a description of the kit contents and guidelines, recommendations and procedures for performing transfections using the DeliverX Peptide Kit.

DeliverX Peptide Transfection Kit Contents and Storage

Contents and Storage of DeliverX Kit

DeliverX Peptide Transfection Kit Contents

Component	Storage
DeliverX Peptide Transfection Reagent	–20 °C
DeliverX Peptide Buffer-1	RT
DeliverX Peptide Buffer-2	RT

Storage Recommendations

- Divide transfection reagent into aliquots to minimize freeze/thaw cycles.
- Store components at recommended temperatures. Product shelf life is 6 months from date of shipment, if stored properly.

Safety Warnings and Precautions

CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

For research use only. Do not use internally or externally in humans or animals.

Required and Recommended Materials Not Provided

Materials Required But Not Provided

Item	Source
Cell culture reagents and equipment	Major laboratory suppliers (MLS)
Phosphate buffered saline, pH 7.2 (PBS)	Invitrogen (P/N 70013-032)
Hank's balanced salt solution	Invitrogen (P/N 14025)
Ultrasonic cleaner (bath sonicator) with 30-40 kHz sonication	Panomics, Inc. (P/N DX0400) or equivalent

Materials Recommended But Not Provided

Item	Source
TAMRA-labeled Peptide Control	Panomics, Inc. (P/N DX1100)
Pre-Formed Peptide Transfection Complex Control	Panomics, Inc. (P/N DX1101)
Airfuge ultracentrifuge	Beckman Coulter (Airfuge)
Fluorescent microscope	Olympus (Model IX71)
20X objective lens, Numerical aperture = 0.75	Olympus (P/N 1-U2B825)
TAMRA filter cube, Ex/Em = 542/568 nm	Omega Optical (P/N XF-32)
#1.5 coverglass base, optical bottom, black, 96-well microplate	NUNC (P/N 164588)
Ammonium bicarbonate	MLS
Acetic acid	MLS
DMSO	MLS

Assay Workflow and Recommended Guidelines, Controls and Optimization

Assay Workflow

	Time Required
1. Prepare Adherent Cells a. Plate cells before transfection	Variable, typically 16-24 hr
2. Prepare Transfection Complex a. Prepare Peptide-Cargo/Buffer-1 b. Prepare Peptide Transfection Reagent/Buffer-2 c. Combine Peptide-Cargo/Buffer-1 and peptide Transfection Reagent/Buffer-2 d. Incubate complex at RT for 30 min	30 min
3. Transfect Cells a. Add complete media (or serum-free media), incubate 2-4 hr at 37 ° C, 5% CO_2 b. Add complete media, incubate 24-72 hr at 37 ° C, 5% CO_2	24-72 hr
4. Quantify Delivery by Fluorescence Microscopy or Functional Assay	Variable, depending on assay

Transfection Guidelines

- Use healthy cells in mid-log phase of growth, not overgrown.
- Use cells between 4–20 passages (passage number is cell-type specific).
- Do not use antibiotics during transfection process. You can use antibiotics to maintain the cell line.
- Sonicate the transfection reagent as stated in the procedure to ensure proper formation of the DeliverX/peptide transfection reagent complexes.

Note Once the DeliverX/peptide transfection reagent complex has been formed properly, it can be diluted to obtain varying peptide concentrations.

- Perform all procedures in a laminar-flow hood using proper tissue culture techniques.

Recommended Controls

Proficiency Controls

For new users or when working with a new cell type, we recommend the use of the following proficiency controls:

- DeliverX/TAMRA-labeled pre-formed peptide to determine if DeliverX Peptide Reagent is compatible with the cell line.
- TAMRA-labeled peptide to assess the complex formation and transfection procedure.
- Fluorescent-labeled peptide cargos to determine the transfection efficiency. However, note that conjugating fluorescent dye may impact the delivery of peptide into cells.

Routine Controls

These controls assess the transfection and peptide knockdown efficiency and we recommend they be used routinely.

- Positive control peptide to ensure that the assay is working in a reproducible manner. This is a validated, high-potency peptide whose functional response is known.
- Cells only to serve as a mock-transfection designed to monitor for any non-transfection related phenomenon during the experiment. This control contains the transfection buffers but does not include either the transfection reagent or peptide cargos.

Note DeliverX Peptide transfection reagent alone should not be used as a negative control because of its high affinity for the cell membrane, uncomplexed reagent might be cytotoxic.

Optimizing Transfections

Because cell types can differ significantly with respect to their capacity to be transfected, we recommend that you optimize the protocol empirically. The most important parameters for optimization are cell density and peptide concentration.

Cell Density

Cells that are too dense or too sparse may not take up an optimal amount of the complexes resulting in minimal effect on functional phenotypes or elevated levels of cytotoxicity.

Peptide Concentration

A peptide's capacity for eliciting a phenotypic response is influenced in part by the peptide design, stability, and nature of its interaction with the target. If too much peptide is used during transfection, you may see toxicity. If too little is used, you may not see an adequate response. The maximum peptide concentration that DeliverX Peptide Reagent can deliver is 1.3 µM (final concentration). A good starting range for peptide concentration is 0.5–1.3 µM.

Recommendation for Optimizing Cell Density and Peptide Concentration

We recommend you perform a two parameter matrix experiment that includes 3 different concentrations (for example, 0.5, 1, and 1.3 µM final concentration) and 3 cell densities (for example, 60, 75, and 90% confluency) and select the cell density and peptide concentration that yields the best cell viability and biological response.

Recommendations for Peptide Design and Preparation

Peptide Design

In general, peptides with the following characteristics are efficiently delivered into most cell types. However, even petides with the following characteristics might not be efficiently delivered into the cells.

DeliverX Peptide Transfection Kit is well-suited for peptides with the following characteristics. Loading capacity (amount of peptide that enters the cell) increases with increasing number of these characteristics.

- Longer peptides (>10 amino acids) are preferred.

- More than one hydrophobic region consisting of a minimum of 3 continuous or alternating aromatic and aliphatic amino acids. Amino acids with the highest hydrophobicity are best. For example, xxxHHHxxx, or xHxHxHxxx where H = hydrophobic amino acid. See below for more information.
- More than one charged region (at pH 7.2) consisting of 3 continuous or alternating negatively and positively charged amino acids. For example, xxxCCCxxx, or xCxCxCxxx where C = positively or negatively charged amino acid at pH 7.2. Positively charged peptides are preferred over negatively charged peptides.
- At least 3 or more Arg or Lys amino acids at the C or N terminus.
- Completely soluble in 1X PBS, pH 7.2.

Amino Acid	**Property**
Phe, Trp, Tyr	Highly hydrophobic (aromatic)
Lle, Leu, Gly, Val, Ala	Moderately hydrophobic (aliphatic)
Arg, Lys, His	Positively charged at neutral pH
Asp, Glu, Gln, Asn	Negatively charged at neutral pH
Pro, Met, Cys, Gly	Neutrally charged at neutral pH

For more information on peptide properties, visit:

- www.innovagen.com

For more information on amino acid properties, visit:

- web.indstate.edu/thcme/mwking/amino-acids.html
- www.mcb.ucdavis.edu/courses/bis102/AAProp.html

Preparing Peptide Cargos

Peptides must be soluble and contain no aggregates. Use the following guidelines for dissolving peptides:

If the peptides are...	**Then...**
Negatively charged and more than 25% of the amino acids are charged	Dissolve in 0.1 M ammonium bicarbonate, then dilute with 1X PBS. **Note** Use the smallest amount of ammonium bicarbonate possible to minimize the impact on cell physiology.
Positively charged and more than 25% of the amino acids are charged	Dissolve in 25% acetic acid, then dilute with 1X PBS. **Note** Use the smallest amount of acetic acid possible to minimize the impact on cell physiology.

If the peptides are...	Then...
Positively or negatively charged but fewer than 25% of the amino acids are charged	Dissolve in 100% DMSO. Add DMSO drop-wise with constant agitation. Sonicate to ensure complete dissolution. Dilute with 1X PBS. **Note** Complex formation is not affected by up to 3% DMSO. **Note** Use the smallest amount of DMSO possible to minimize the impact on cell physiology.

- Remove aggregates by sonication for 5 minutes followed by centrifugation at 200,000 x g for 30 minutes. We recommend the AirFuge Ultracentrifuge from Beckman Coulter. Microcentrifuges that generate 14,000 x g can also be used but might not produce optimal results.
- Store dissolved peptide at –80 °C.

Preparing the Cells the Day Before Transfection

Adherent Cell Types

For most adherent cell types, the optimal confluency for transfection is 60–90%. The following table provides guidelines for seeding differently sized culture vessels to obtain 60–90% confluence after 24 hours of growth.

IMPORTANT Use cells from passages 4–20 (cell-type specific). Optimal cell density is very important for obtaining the best results.

Note For experiments in which you will be transfecting fluorescently-labeled peptides (such as TAMRA-labeled peptide control) and viewing the cells under a microscope, we recommend that you grow the cells on a #1.5 coverslip or glass-bottom microplate for optimal detection of fluorescent signal.

If you are using a...	Then seed cells at a density of...	In a volume of...
6-well plate	150–300 x 10^3 cells/well	2 mL/well
12-well plate	50–200 x 10^3 cells/well	1 mL/well
24-well plate	25–75 x 10^3 cells/well	500 µL/well
96-well plate	5–10 x 10^3 cells/well	100 µL/well

Note These numbers are approximate because the exact number of cells required depends on cell type, size, and growth rate.

Preparing the Peptide/Peptide Transfection Reagent Complex

About Preparing the Complex

Sonication of the DeliverX Peptide Transfection Reagents per the procedure below is critical for proper formation of the complex used for transfection. Once the complex has been properly formed, it is amenable to dilution for evaluating a range of peptide delivery concentrations. This novel peptide-based delivery system requires no

optimization of the peptide to transfection reagent ratio and enables high efficiency peptide transfection typically with 1.3 µM or less.

Procedure

The following procedure prepares transfection complexes sufficient for the transfection of 1 well of a 6-, 12-, 24-, or 96-well plate.

To prepare DeliverX peptide/peptide transfection reagent complex:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Thaw peptides and DeliverX Peptide Transfection Reagent and store on ice.</td></tr>
<tr><td>2</td><td>Prepare peptide working stocks:
a. Dilute the peptides to 160 µM with 1X PBS
b. Sonicate in the water bath sonicator at maximum output and power for 5 minutes. If peptides have aggregates or precipitates, centrifuge as described in “Preparing Peptide Cargos” on page 9.</td></tr>
<tr><td>3</td><td>Dilute the 160 µM peptide working stocks with Buffer-1 in 1.5 mL tubes as described in the table below.

<table>
<tr><th>Per Well of a...</th><th>160 µM Peptide Working Stocks (µL)</th><th>Buffer-1 (µL)</th></tr>
<tr><td>6-well plate</td><td>15</td><td>60</td></tr>
<tr><td>12-well plate</td><td>10</td><td>40</td></tr>
<tr><td>24-well plate</td><td>7.5</td><td>30</td></tr>
<tr><td>96-well plate</td><td>1.5</td><td>6</td></tr>
</table></td></tr>
<tr><td>4</td><td>Prepare the DeliverX Peptide Transfection Reagent:
a. Sonicate the DeliverX Peptide Transfection Reagent at maximum output and continuous power for 3–5 minutes to achieve a homogenous solution.
b. Prepare dilutions in 1.5 mL tubes as described in the table below.

IMPORTANT Sonication of DeliverX Peptide Transfection Reagent is critical for achieving good complex formation. Ensure that the tubes are submerged in the water during sonication.

<table>
<tr><th>Per Well of a...</th><th>DeliverX Peptide Transfection Reagent (µL)</th><th>Buffer-2 (µL)</th></tr>
<tr><td>6-well plate</td><td>60</td><td>15</td></tr>
<tr><td>12-well plate</td><td>40</td><td>10</td></tr>
<tr><td>24-well plate</td><td>30</td><td>7.5</td></tr>
<tr><td>96-well plate</td><td>6</td><td>1.5</td></tr>
</table>
c. After diluting, vortex briefly and sonicate again at maximum output and continuous power for 3–5 minutes.</td></tr>
</table>

To prepare DeliverX peptide/peptide transfection reagent complex: *(continued)*

Step	Action
5	Form concentrated peptide-cargo/peptide transfection reagent complex: a. Mix the peptide-cargo/Buffer-1 solution from Step 3 with the Peptide Transfection Reagent/Buffer-2 solution from Step 4. b. Incubate tubes at RT for 30 minutes. c. Prepare complex dilution buffer by mixing equal volumes of Buffer-1 and Buffer-2. d. Add an equal volume of complex dilution buffer to the peptide/peptide transfection complex. The concentration of peptide is 8 µM at this point. Without further dilution, final peptide concentration, after addition of growth media, will be 1.3 µM. To make lower concentrations, dilute using complex dilution buffer. **Note** After incubation, the concentrated peptide/peptide transfection reagent complex can be stored at –20 °C for up to 6 months. The concentrated complex is stable for up to 10 freeze/thaw cycles.

Transfecting Adherent Cells

Assay Preparation

Pre-warm both serum-free and complete growth media to 37 °C.

Procedure

To transfect adherent cells:

Step 1

Carefully, remove media from the wells and wash once with PBS.

Per Well of a...	1X PBS (µL)
6-well plate	500
12-well plate	300
24-well plate	150
96-well plate	100

Step 2

Add the working peptide/peptide transfection reagent complex.

Per Well of a...	Working DeliverX Peptide/Peptide Complex (µL)
6-well plate	300
12-well plate	200
24-well plate	150
96-well plate	30

IMPORTANT Manually rock (do not swirl) the plate to evenly distribute the complex over the well surface.

Step 3

Add complete growth media and mix by gentle rocking.

Note Peptide concentration, if undiluted, is 2.6 µM.

Per Well of a...	Complete Growth Media (µL)
6-well plate	600
12-well plate	400
24-well plate	300
96-well plate	60

To transfect adherent cells: *(continued)*

Step	Action
4	Incubate under normal cell-culture conditions, typically at 37 °C and 5% CO_2, for 4 hours. Proceed to the next step. **Note** For maximal loading, we recommend 4 hours. (Optional). For some peptides and cell types, delivery efficiency and loading capacity increase by using serum-free media instead of serum-containing media. For cells transfected with fluorescent-labeled peptide: a. Incubate under normal cell-culture conditions for 0.5–4 hours. b. Carefully wash the cells twice with pre-warmed PBS. c. Add Hank's Balanced Salt solution. For extensive viewing, use Hank's solution plus 2% serum. d. Examine and image the cells using a fluorescent microscope. **Note** Use TAMRA filter (Ex/Em = 542/568) for TAMRA-Peptide Control or Pre-Formed Control.
5	Add complete growth media. At this step, the final concentration of peptide in the sample is 1.3 µM without dilution of the working stock DeliverX/peptide complex.
6	Incubate under normal cell-culture conditions, typically at 37 °C and 5% CO_2 for 24 hours or desired time intervals. For example, the induction of cell cycle arrest using CDK2 inhibiting peptide requires analysis after 24–48 hours of incubation after the peptide/transfection complex addition. However, the induction of ERK1/2 phosphorylation by G gamma protein inhibiting peptide requires analysis within 15 minutes of incubation after the peptide/transfection complex addition.

Per Well of a...	Complete Growth Media (µL)
6-well plate	900
12-well plate	600
24-well plate	450
96-well plate	90

Transfecting Cells in Suspension (Reverse Transfection)

About Transfecting Cells in Suspension

This procedure describes the transfection of adherent cells after trypsinization and while they are in suspension. Transfect adherent cells in suspension when you want to perform high-throughput screening of many peptides on a single cell type.

Assay Preparation

- Grow adherent cells so that they are 70–90% confluent on the day of transfection.
- Pre-warm both serum-free and complete growth media to 37 °C.

Procedure

To transfect cells in suspension:

Step	Action
1	Trypsinize and pellet cells by centrifugation.

To transfect cells in suspension: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Gently resuspend cells in PBS using a wide-bore pipet and pellet again.</td></tr>
<tr><td>3</td><td>Gently aspirate the media, avoiding cell loss, and resuspend the cells in PBS to the concentration as indicated in the table below.

<table>
<tr><th>For a...</th><th>Resuspend to...(cells/mL)</th></tr>
<tr><td>6-well plate</td><td>3.0–9.0 x 10^6</td></tr>
<tr><td>12-well plate</td><td>1.0–4.0 x 10^6</td></tr>
<tr><td>24-well plate</td><td>1.0–3.0 x 10^6</td></tr>
<tr><td>96-well plate</td><td>0.5–1.0 x 10^6</td></tr>
</table></td></tr>
<tr><td>4</td><td>Gently pipet the cell suspension into the plate as indicated in the table below:

<table>
<tr><th>Per Well of a...</th><th>Add... (µL) of the Cell Suspension</th></tr>
<tr><td>6-well plate</td><td>100</td></tr>
<tr><td>12-well plate</td><td>50</td></tr>
<tr><td>24-well plate</td><td>25</td></tr>
<tr><td>96-well plate</td><td>10</td></tr>
</table>
Note For experiments in which you will be transfecting fluorescent-labeled peptide (such as TAMRA-labeled peptide control) and viewing the cells under a microscope, we recommend that you grow the cells on a #1.5 coverslip or glass-bottom microplate for optimal detection of fluorescent signal.</td></tr>
<tr><td>5</td><td>Add the working DeliverX peptide/peptide transfection reagent complex as indicated in the table below:

<table>
<tr><th>Per Well of a...</th><th>Working DeliverX Peptide/Peptide Complex (µL)</th></tr>
<tr><td>6-well plate</td><td>300</td></tr>
<tr><td>12-well plate</td><td>200</td></tr>
<tr><td>24-well plate</td><td>150</td></tr>
<tr><td>96-well plate</td><td>30</td></tr>
</table></td></tr>
<tr><td>6</td><td>IMPORTANT Manually rock (do not swirl) the plate to evenly distribute the complex over the well surface.</td></tr>
<tr><td>7</td><td>Immediately add complete growth media as indicated in the table below and mix by gently rocking.

<table>
<tr><th>Per Well of a...</th><th>Complete Growth Media (µL)</th></tr>
<tr><td>6-well plate</td><td>600</td></tr>
<tr><td>12-well plate</td><td>400</td></tr>
<tr><td>24-well plate</td><td>300</td></tr>
<tr><td>96-well plate</td><td>60</td></tr>
</table></td></tr>
</table>

To transfect cells in suspension: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>8</td><td>Incubate under normal cell-culture conditions, typically at 37 °C and 5% CO_2, for 4 hours. Proceed to the next step.

Note For maximal loading, we recommend 4 hours.

(Optional). For some peptides and cell types, delivery efficiency and loading capacity increase by using serum-free media instead of serum-containing media.

For cells transfected with fluorescent-labeled peptide:
a. Incubate under normal cell-culture conditions for 0.5–4 hours.
b. Carefully wash the cells twice with pre-warmed PBS.
c. Add Hank’s Balanced Salt solution. For extensive viewing, use Hank’s solution plus 2% serum.
d. Examine and image the cells using a fluorescent microscope.

Note Use TAMRA filter (Ex/Em = 542/568) for TAMRA-Peptide Control or Pre-Formed Control.</td></tr>
<tr><td>9</td><td>Add complete growth media. At this step, the final concentration of peptide in the sample is 1.3 µM without dilution of the working stock DeliverX/peptide complex.

<table><tr><th>Per Well of a...</th><th>Complete Growth Media (µL)</th></tr><tr><td>6-well plate</td><td>900</td></tr><tr><td>12-well plate</td><td>600</td></tr><tr><td>24-well plate</td><td>450</td></tr><tr><td>96-well plate</td><td>90</td></tr></table></td></tr>
<tr><td>10</td><td>Incubate under normal cell-culture conditions, typically at 37 °C and 5% CO_2 for 24 hours or desired time intervals.

For example, the induction of cell cycle arrest using CDK2 inhibiting peptide requires the analysis after 24–48 hours of incubation after the peptide/transfection complex addition. However, the induction of ERK1/2 phosphorylation by G gamma protein inhibiting peptide requires the analysis within 15 minutes of incubation after the peptide/transfection complex addition.</td></tr>
</table>

Troubleshooting

Problems and Recommended Actions

To troubleshoot transfections using DeliverX Peptide Transfection kits:

Observation	Possible Cause	Recommended Action
Expected phenotype is not observed	Peptide is unable to form transfection complex due to one or more of the following: ♦ Not soluble ♦ Does not contain hydrophobic domains ♦ Does not contain positive or negative charged domains ♦ Less than 10 amino acids in length	Follow the guidelines in "Recommendations for Peptide Design and Preparation" on page 8.
	Non-functional peptide	Redesign peptide for increased binding affinity to the target protein or test additional peptides.
	Cell density not optimal	Evaluate cell densities outside of the recommended 60–90% confluence at the time of transfection.
	Transfection time not adequate	Evaluate transfection times greater than 4 hours.
	Cell have a tendency to grow in groups or clumps	Do not tap flask during trypsinization. Allow the cells to detach themselves. After trypsinizing the cells, pipet up and down several times to release the cells. Seed cells at the desired density. Visually check cell density using a light microscope.
	Cell response changes after repeated passages	Thaw fresh cells for subsequent experiments. Avoid using cells at early or late passages.
	Serum interferes with delivery	Reduce the amount of serum or use serum-free media as described in the procedure.
High toxicity	Cell density too low	Evaluate higher cell densities at the time of transfection. Be gentle when removing medium or PBS during washes.
	Cells become more sensitive to reagents after repeated passages	Thaw fresh cells for subsequent experiments. Avoid using cells at early or late passages.
	Too much working transfection complex added to the cells	Follow the recommended guidelines stated in the manual for optimizing transfection.

To troubleshoot transfections using DeliverX Peptide Transfection kits: *(continued)*

Observation	Possible Cause	Recommended Action
Peptide aggregates	Peptides are not completely soluble	Dissolve in desired solvent. See "Required and Recommended Materials Not Provided" on page 6 for more information. Ultra-centrifuge to remove aggregates.
Non-diffuse delivery pattern of fluorescent peptide	Peptides stick to plate	Avoid using collagen, matrigel, fibronectin, or poly-lysine coated plates. Switch to another plate type.
	Peptide cargo contains insoluble aggregate	Sonicate and centrifuge peptide cargo before mixing with DeliverX peptide.
Unable to observe fluorescent-labeled peptide	Sensitivity of the microscope setup is poor.	Grow cells on 170 µm thick (#1.5) glass substrate such as a standard glass cover slip or glass-bottom microwell. Use a microscope with high Numerical Aperture lens and fluorescent filter setup. See "Required and Recommended Materials Not Provided" on page 6.
	High background due to peptides or DeliverX/Peptide complex binding non-specifically to cell culture plate or substrate	Switch to a different culture vessel type or format. Avoid using collagen, matrigel, fibronectin, or poly-lysine coated plates.
	Signal masked by auto-fluorescent cells	Use fluorophores that excite and emit at the range of 500 nM and 600 nM respectively, for example, TAMRA or Rhodamine. Minimize the usage of fluorophores that require 365–488 nm excitation wavelength, for example, FITC, Dapi, or Coumarin.

Appendix I: Delivery of Polar Peptides

About this Appendix

This section briefly describes the experimental details that were used to perform transfections using the DeliverX Peptide Transfection Kit and summarizes the results in tabular format.

Experimental Conditions

HeLa cells were seeded at 7,500 cells/well into a #1.5 glass-bottom 96-well plate. The next day 1.3 µM FITC-labeled peptide complex was added to each well followed by the addition of serum-free media. Cells were incubated for 2 hours and then complete growth media was added. Cells were incubated for an additional 2 hours before they were gently washed twice with PBS. Complete growth media was added and cells were viewed under the microscope to determine transfection efficiency, loading, and viability index. Similar experiments were performed on NIH3T3 cells.

In general, transfection efficiency was greater than 70% when the loading index (see “Scale of Loading Index” on page 20 for a definition of this metric) was greater than 3.

Position and intensity of hydrophobic, polar, and neutral regions were analyzed using peptide property calculation software provided at www.innovagen.com.

Results

Sequence[a]	Length	Hydrophillic Residues (%)	MW[b]	Peptide Charge	Loading Index[c]	Viability Index[d]
GIKNNLKDCGLD	12	50	1794.38	0	3	5
GIKNNLKECGLT	12	45	1793.38	+1	2	5
CGLHDNLKQLMLQ	13	38	2019.38	+1	1	4
GNGIKCLFNDKL	12	42	1826.38	+1	5	4
CGGRMAPPRRDAMPSDA	17	35	2293.38	+1	5	5
SIRKALNILGYPDYD	15	40	2243.38	0	3	5
MPKKKPTPIQLNP	13	38	1997.38	+3	5	5
KGRKPRDLELPLSPS	15	53	2197.38	+2	3	5
GSFLVRES	8	50	1398.38	0	2	5
RFARKGALRQKN	12	58	1950.38	+5	3	5
RFAAKGALRQKN	12	50	1865.38	+5	5	5
PVKRRLFG	8	38	1477.38	+3	4	5
PVKRRLDL	8	50	1501.38	+2	4	5
PVKRRLFL	8	38	1533.38	+3	2	5
DAAREGFLDTAVVAHRAGAR	20	30	2587.38	+1	5	5
CTMNRRGAIKQAK	13	46	1982.38	+4	4	4
CTMNARGAIKQAK	13	38	1897.38	+3	2	5
CPRKRQGAVRRRV	13	54	2087.38	+6	5	4
CPRKAQGAVRRRV	13	48	2002.38	+5	3	5
CTRKRQRAMRRRV	13	62	2223.38	+7	5	5
CTRKAQRAMRRV	13	54	2138.38	+6	5	5

Sequence[a]	Length	Hydrophillic Residues (%)	MW[b]	Peptide Charge	Loading Index[c]	Viability Index[d]
CSIYRRGARRWRK	13	54%	2213.38	+6	5	5
CSIYARGARRWRK	13	46%	2127.38	+5	5	5
GRAGNQYL	8	38%	1383.38	+1	3	4
GGLPPFRAG	9	11%	1376.38	+1	1	5

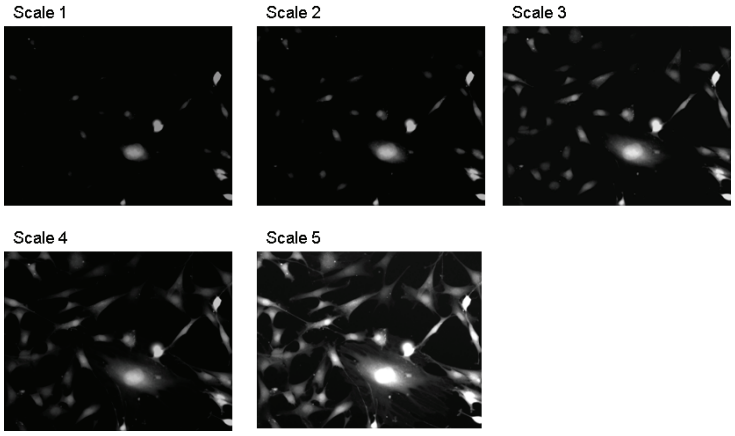
a. Peptides consist of FITC and aminohexanoic acid linker at the N-terminus and amide modification at the C-terminus.

b. Molecular weight of peptides, linker, and FITC

c. Loading index = qualitative measurement using intensity of FITC signal in cells to determine the relative amount of peptide that enters the cell (see images below).

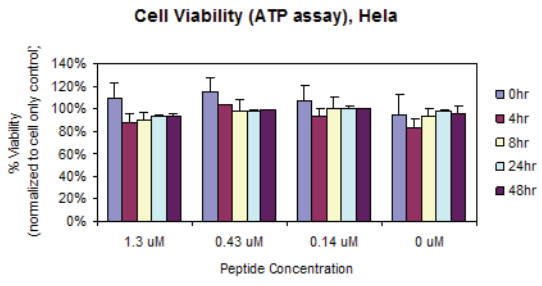
d. Viability index = qualitative measurement using cell density and morphology to estimate viability. For more information, see ATP viability assay below.

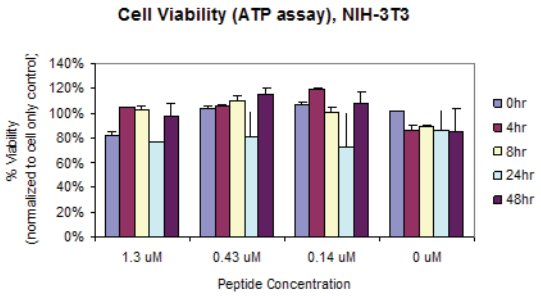
Scale of Loading Index



HeLa cells transfection with TAMRA-labeled peptide control.

ATP Viability Assay

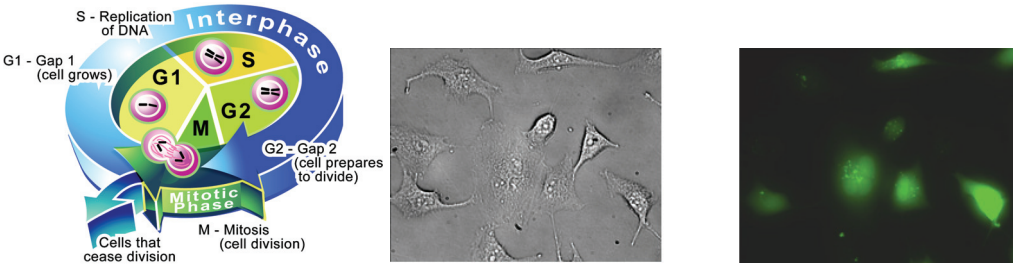




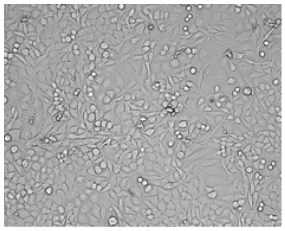
Peptide/Transfection Complex was added to cells and ATP levels were measured at 0, 4, 8, 24, and 48 hours.

Functional Assay

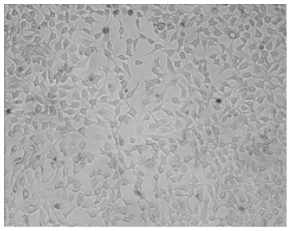
Inhibition of Cell Proliferation by Cdk2 Peptide in HeLa Cells



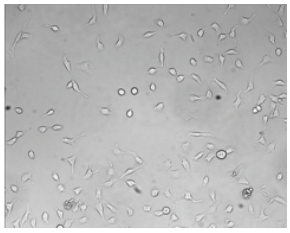
Left: Cartoon of the cell cycle[1]. Right: HeLa cells transfected with FITC-labeled Cdk2 peptide.



Cells only, at 48 hrs.



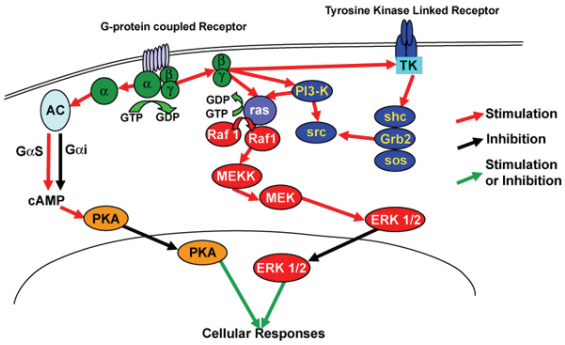
DeliverX Peptide Transfection Reagent + Negative Control at 48 hr.



DeliverX Peptide Transfection Reagent + Cdk2 peptide at 48 hr.

HeLa cells grown in 96 well microplate were transfected with Cdk2 inhibiting peptide using DeliverX Peptide Transfection Reagent and incubated for 48 hours before the brightfield images were obtained.

G_β Binding Peptide Induces Phosphorylation of Erk 1/2 in Rat 2 Fibroblast



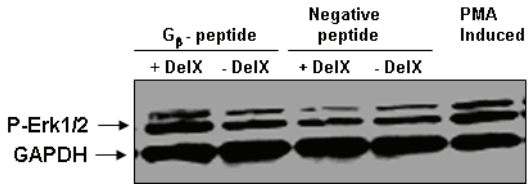
Flow chart illustrating the Known G-protein activation of ERK 1/2 pathways

G_β peptide (SIRKALNILGYPDYD) binds to G-Beta/Gamma subunit and induces the dissociation of G-alpha subunit[2]. The associated G-Beta/Gamma subunit activates the MAPK pathway resulting in increased phosphorylation of ERK 1/2[3].

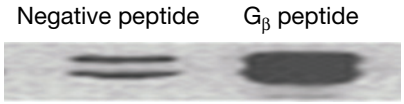
1. Image courtesy of *The Science Creative Quarterly* (www.scq.ubc.ca) Jane Wang, illustrator.

2. *J Biol Chem*, 2003, Vol 278: 19634-19641

3. Image courtesy of www.vascularweb.org



G_β peptide was transfected into Rat2 cells using DeliverX Peptide Transfection Reagent. Rat 2 cells were lysed 15 minutes after transfection and western blotting was performed to measure phosphorylation of ERK 1/2. GAPDH is shown as a control for loading.



Results of a similar experiment performed by Goubaeva *et al*. using myristoylated G_β peptide[2].

Contacting Panomics

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