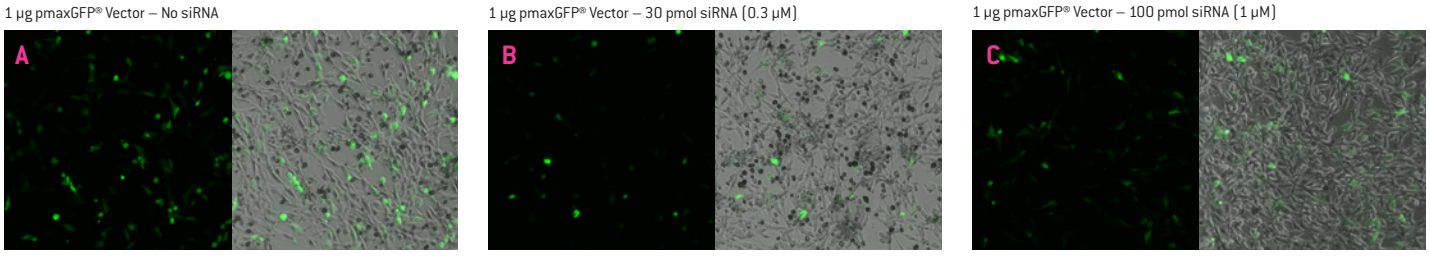
Amaxa® siRNA Test Kit

For Cell Lines and Adherent Primary Cells

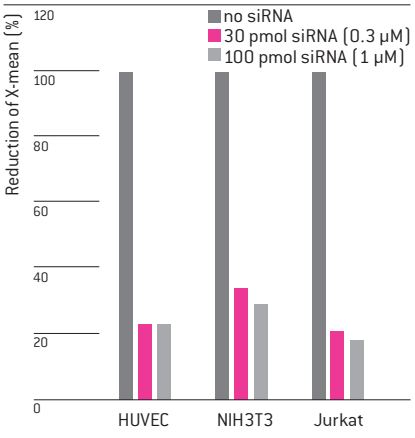
Kit principle This kit has been designed to enable an easy set-up of siRNA transfections in your cells of interest by co-transfection of pmaxGFP® Vector, encoding the green fluorescent protein (GFP) from *Pontellina p,* with an siRNA directed against maxGFP® Protein. Successful gene silencing is monitored as decrease of green fluorescence compared to control sample using fluorescence microscopy. In subsequent siRNA experiments, the kit can be used as a positive control.

Note **The kit is not suitable for use with primary blood cells for which we recommend an alternative protocol.**

Example of siRNA mediated gene silencing of maxGFP® Protein Expression



NIH3T3 cells (ATCC® CRL-1658™) were transfected with 1 µg of pmaxGFP® Vector alone (A) or in combination with 30 or 100 pmol siRNA (B, C) targeting maxGFP® mRNA. Gene silencing of maxGFP® Protein Expression was monitored by fluorescence microscopy 24 hours post Nucleofection®. Right pictures show bright field overlaid with fluorescence.



NIH3T3 cells [ATCC® CRL-1658™], Jurkat clone E6.1 [ATCC® TIB-152™] or primary Clonetics® HUVEC cells were transfected with 1 µg of pmaxGFP® Vector alone or in combination with 30 or 100 pmol siRNA targeting maxGFP® mRNA. Gene silencing of maxGFP® Protein Expression was measured 24 hours post Nucleofection® by flow cytometry. The x-mean value of each siRNA sample was normalized to the control sample transfected with pmaxGFP® Vector alone (set to 100%). The x-mean value represents the average fluorescence intensity per cell, i.e. the average maxGFP® Expression Level per cell.

Product Description

Cat. No.	**VSC-1001**
Size (reactions)	35 – 150* reactions
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	2 x 30 µg
siRNA targeting maxGFP® mRNA (lyophilized)	5 nmol

*Number of total reactions depends on used Nucleofection® Volume (Nucleofector® Device: 100 µl; 96-well Shuttle® Device: 20 µl)

Storage and stability	Store all reagents at -20°C. Repeated freeze-thaw cycles will not interfere with the siRNA sample (dissolved or lyophilized) as long as RNAse-free conditions are strictly maintained. After thawing, spin the tube briefly, to collect content at the bottom of the tube. The expiry date is printed on the kit label.

Kit Strategy

A simple strategy to set up siRNA in your cell of interest

– **Step1:** Choose the appropriate Nucleofector® Kit and corresponding Optimized Protocol for your cell line or primary cell of interest. If no Optimized Protocol is available for your cell line of interest, please optimize Nucleofection® Conditions first using our Cell Line Optimization Kits (VCO-1001 or VHCO-1001). In case a protocol is lacking for your primary cell of interest, please contact our Scientific Support Team for further advice.

Note **With the Nucleofector® Technology, siRNA and DNA are transfected using the same conditions, i.e. Nucleofector® Solution and Program.**

Note **Generally, it is not advisable to optimize Nucleofection® Conditions using fluorescently labeled siRNA duplexes. Unless analyzed by confocal fluorescence microscopy, exact transfection efficiencies are difficult to determine**

– **Step2:** Confirm that the RNAi mechanism is working in your cell type of interest by using the siRNA Test Kit in combination with the optimal Nucleofections® Conditions

Note **The easiest way to monitor gene silencing of maxGFP® Protein is by fluorescence microscopy. Flow cytometry can be used for quantitative analysis. In this case, gene silencing of maxGFP® Protein is often easier to detect by monitoring the decrease in the mean fluorescence intensity (reflecting the protein level per cell) instead of transfection efficiency (reflecting the number of expressing cells)**

– **Step3:** Once the gene silencing properties of your cell type of interest are confirmed you can establish gene silencing conditions of your specific gene of interest. The siRNA Test Kit can be further used as positive control for transfection and knockdown.

Experimental set-up for the siRNA Test Kit

– As the RNAi mechanism varies with every cell type, we recommend testing a fixed amount of pmaxGFP® Vector together with two different amounts of siRNA oligonucleotide in an initial experiment (amounts refer to a 100 µl Nucleofection® sample, when using a 20 µl format use 1/5 of the indicated amounts):

	Sample 1 (reference)	**Sample 2**	**Sample 3**	**Sample 4** (negative control)
pmaxGFP® Vector	1 µg	1 µg	1 µg	1 µg
siRNA	–	30 pmol	100 pmol	100 pmol Unspecific siRNA*

*Any available unspecific siRNA (e.g. targeting GAPDH or vimentin) can be used as negative control

Required Material

- Nucleofector® Device or Nucleofector® 96-well Shuttle System
- Cell-type specific Nucleofector® Kit and Protocol
- Supplied pmaxGFP® Vector
- Supplied siRNA oligonucleotide
- **For siRNA resuspension:** Sterile RNase-free water or siRNA suspension buffer (100 mM KOAc, 30 mM HEPES-KOH, 2 mM MgOAc, pH 7.4)

1. Pre Nucleofection®

Cell culture

1.1 For detailed information on cell culture conditions and cell numbers please refer to the cell-type specific Nucleofection® Protocol or contact Lonza's Scientific Support Team for further assistance.

Preparation of siRNA stock

1.2 Resuspend lyophilized siRNA (5 nmol) in 250 µl RNase-free water or siRNA suspension buffer to obtain a 20 µM siRNA stock solution.
1.3 Heat the tube to 90°C for 1 min
1.4 Incubate at 37°C for 60 min
1.5 If not used directly store siRNA stock at -20°C

2. Nucleofection®

One Nucleofection® Sample contains

	100 µl (standard cuvette)	20 µl (Nucleocuvette® Well)
Cell number	$2 \times 10^5 - 5 \times 10^6$ cells*	$4 \times 10^4 - 1 \times 10^6$ cells*
pmaxGFP® Vector	1 µg	200 ng
siRNA	30 or 100 pmol (i.e. 1.5 or 5 µl of 20 µM stock)	6 or 20 pmol (i.e. 1.2 or 4 µl of 20 µM stock diluted 1:4)
Nucleofector® Solution	100 µl	20 µl

*See cell-type specific Optimized Protocol

2.1 For a description of the Nucleofection® Procedure, please refer to the cell-type specific Optimized Protocol available at **www.lonzabio.com/protocols**
2.2 At the respective step add indicated amounts of pmaxGFP® Vector and siRNA to the cells suspended in Nucleofector® Solution

3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis. Analyze gene silencing after 24 hours by fluorescence microscopy or flow cytometry

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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