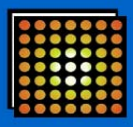

Leopard™ Transfection Array v1.0

A new leap in transfection technology

Product: ***Leopard*** **™ Transfection Array v1.0**
Catalog #: **CLA4404 (array) and CLI4380-4398 (individual polymers)**

FOR RESEARCH USE ONLY

Physical Description:

The *Leopard* Transfection Array v1.0 is packaged in two configurations:

a. *Leopard* Transfection Array v1.0 kit (CLA4404, includes three 96-well plates containing *Leopard* individual polymers in DMSO and one β-gal reporter plasmid vial containing plasmid in acetate buffer pH 5.0)
b. *Leopard* Transfection Array v1.0 individual polymers (CLI4380-CLI-4398, 3 ml each @ 20mg/ml, equivalent to the concentration provided for a 200:1 P:D ratio in CLA4404)

Storage and Care:

Store all *Leopard* Transfection Array v1.0 plates and *Leopard* Transfection Array v1.0 individual polymers at 4°C for up to one year. To avoid loss of polymer solution in plates upon removing foil seal, always store plates label-side up. If plate is rotated and the polymer/DMSO solution is allowed to thaw (DMSO m.p. = 23°C), spin the plate in a 96-well plate centrifuge before removing the seal.

I. Product Description

a. Overview

The *Leopard* Transfection Array v1.0 provides you with 23 proprietary polymers in a convenient, economical format to enable the identification of effective polymers for transfecting your particular cell line. The polymers are poly(β-amino esters) systematically synthesized and validated for transfection of mammalian cells. Since it is known that the optimal polymer:DNA (P:D) ratio can be significantly affected by transfection conditions and cell type, the polymers are provided at a range of concentrations. This allows for both the determination of the optimal polymer and the near-optimal P:D for your cell line in a single screening step.

b. Background

The completion of the sequence of the human and other genomes, along with the continually expanding access to cloned genes has caused a parallel increase in the interest and need to introduce gene clones into appropriate cell types in order to gain a more complete understanding of the function of each gene in a relevant biological system. The transfer of cloned genes into eukaryotic cells is often accomplished

through a process known as transfection that employs chemical agents that aid in the internalization of plasmid DNA. Researchers are often confronted with the problem of transfecting cell types (e.g., neural cells, non-immortalized cells or primary cells) that display properties that are vastly different from typical cell lines used in research laboratories. It is frequently a frustrating and expensive challenge to find a transfection reagent and/or protocol that will work in a particular cell type. Often, researchers will define a set of commercially available transfection reagents for screening purposes to find a functional transfection reagent for a new cell type. This approach is expensive and has limited success. The *Leopard* ™ Transfection Array v1.0 provides a more efficient alternative for identifying an appropriate polymer for your transfection needs. In a single screening, 23 different transfection polymers can be evaluated simultaneously, each at four different P:D ratios, to both identify and optimize an appropriate custom transfection reagent for use in your experiments.

II. Kit Contents

1. Included components

   a. *Leopard* Transfection Array v1.0 individual polymers

      23 proprietary transfection polymers are provided in a 96-well format, each at ready-to-use concentrations and volumes.

   b. β-galactosidase reporter plasmid (2ml @ 100 ng/µl in 25mM sodium acetate buffer, pH 5.0)

   c. Instruction Manual

2. Required materials and equipment

   It is assumed that the user has the facilities, equipment and training required for cell culture. Plasmid DNA that includes a β-galactosidase reporter gene is included in this kit for rapid screening using the array. It may be preferable to use some other vector and reporter for testing, such as a specific cloned gene of interest. The user, prior to setting up a transfection screen using this kit, should define appropriate and detailed protocols for the assay of any particular cloned gene.

   Additional materials required to use this product are:

   Growth media for your cells

   Opti-MEM® (Invitrogen)

   Mammalian β-galactosidase assay kit (Pierce cat # 75705)

   *See Appendix for supplier information.

III. Detailed Protocols

NOTE: Transfection is a complex process regardless of the reagent or protocol used. It is important to practice impeccable technique and pay attention to details. The following protocol has been effective for screening COS-1, MO3.13, HEPG2, and HEK293 cells. However, modifications may be necessary for other cells, including primary cells. These may include using different media in the transfection, the density of the cells, or the amount of time for incubation of the cells with the polymer:DNA complexes.

PREPARATION PRIOR TO TRANSFECTION:

Cells: The *Leopard* Transfection Array v1.0 individual polymers are arrayed in a 96-well format for convenience in screening the entire set. Thus, cells should be seeded into 96-well plates. Seeding cells at a density of ~5 X 10^4 cells per well on the day before transfection and incubating them overnight provides cells

that are at a good density and in a good metabolic state for transfection with *Leopard* ™ Transfection Array v1.0 individual polymers. However, it is likely that the optimal seeding density varies with the specific cell type used. After a transfecting polymer (or polymers) has been identified, it may be helpful to experiment with seeding and preincubation conditions to optimize further the transfection conditions for specific cell types and transfection conditions and the effective *Leopard* ™ Transfection Array v1.0 polymer(s).

ON THE DAY OF THE TRANSFECTION: (Note: all of the following steps must be performed without allowing any wells containing cells to dry out.)

1. Examine the cells microscopically. They should be healthy. Place the plate of cells back in the incubator.
2. Remove the *Leopard* ™ Transfection Array v1.0 plate(s) from the refrigerator and allow to warm to room temperature. (Note: the polymers are provided in DMSO and will be solid at temperatures below 18°C. It is important to warm the plate to room temperature before use to provide a liquid solution).
3. Dispense 6µl of plasmid DNA into the wells of the array plate(s) containing the polymers and mix well by pipetting up and down five times.
4. Incubate the plate at 37°C for 1 hour.
5. Remove the array plate from the incubator and add 80µl of Opti-MEM® (prewarmed to 37°C) into each well and mix thoroughly by pipetting up and down five times.
6. Set the array plate aside.

Perform the following steps quickly and working row by row or column by column to avoid drying of cells during the process.

7. Remove the 96-well plate(s) containing the cells from 37°C CO_2 incubator.
8. Remove the media from each well using a multi-channel pipettor.
9. Transfer 80µl of the Opti-MEM®:polymer:DNA solution from the array plate to the cells using a multi-channel pipettor.
10. Incubate the cell plate in a 37°C CO_2 incubator for 3 hours.
11. Carefully pipette off the Opti-MEM®.
12. Add 100µl of the appropriate complete growth media.
13. Incubate in a 37°C CO_2 incubator for 24 to 72 hours.

IV. Analysis of Results and Follow-up Optimization

IDENTIFICATION OF EFFECTIVE TRANSFECTING POLYMERS: The ideal transfection reagent would display high transfection with low toxicity to the target cells. In practice, it is often necessary to accept a compromise between these two properties. To identify the best polymer for your system, assess each well for at least two characteristics. The first of these is cell viability and the second is for expression of the transfected gene.

Cell toxicity. The polymers included in the *Leopard* ™ Transfection Array v1.0 may show mild cytotoxicity depending on the polymer concentration and the cell type. This may result in unacceptable cytotoxicity with some of the polymers in your cell line. However, the diversity of the polymers in the array usually allows for the identification of a desirable reagent. To assess toxicity you can visually assess the cells in each well after transfection or you can use one of a number of kits commercially available for measuring cell viability.

Assessment of transfection of target gene. You are provided with plasmid DNA that includes a β-galactosidase reporter gene for testing the array. If you prefer to use some other vector and reporter for testing, you will also need a plasmid vector containing the DNA sequence in which you are interested. The plasmid vector should be prepared at 100 ng/µl in 25 mM sodium acetate buffer, pH 5.0. You should also have appropriate and detailed protocols for the assay of your particular cloned gene.

Example assay for the β-Galactosidase Reporter using the Pierce Mammalian β-gal Assay Kit (Pierce cat # 75705):

i. Perform a transfection screening as described earlier using a plasmid vector containing the β-gal reporter (provided in the kit).

ii. After allowing for expression for the desired amount of time (24-72h), carefully pipette off the media from each of the wells.

iii. Add the reagents as described in the Pierce β-gal Assay Kit manual (not provided).

iv. During incubation, intermittently visually inspect the plate for any wells that start to show development of yellow color (see Figure 1). Those wells that develop color indicate positive transfection activity for the polymer and P:D ratio. The earlier that color develops, the better the transfection ability of the polymer. This type of analysis is usually sufficient for initial screening. You can assess color development by using a multi-well plate scanner to obtain more quantitative results if desired.

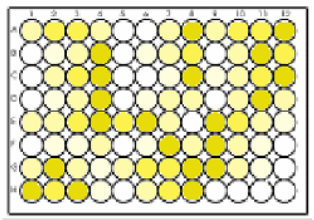

Figure 1. *Leopard* ™ Transfection Array v1.0 screened against HEK293 cells following supplied protocols. Polymers are arranged in groups of four (from left to right, in decreasing P:D for each set). Intensity of color is proportional to amount of amount of b-galactosidase expressed by the cells in that well.

Following the identification of one or more effective *Leopard* ™ Transfection Array v1.0 polymer(s), any single polymer can be ordered (see graphic key for polymer ID #s, Figure 2). Further optimization of the transfection protocol for a particular cell type may also be desirable. Other parameters that may affect the transfection efficiency include the cell density, the amount of plasmid DNA, and the time of exposure of the cells to the polymer:DNA complex, and the media used in the transfection step. Any or all of these parameters can be optimized with the *Leopard* ™ Transfection Array v1.0 individual polymer(s) that have been identified as effective in the target cell line.

Identification of effective polymers and polymer ordering information

Cell transfection results will be affected by the quantity of polymer added and the target cell line. To give you the best initial transfection screening result, all 23 polymer in the *Leopard* Transfection Array v1.0 are provided in sets of four concentrations decreasing from left to right (e.g. A1 to A4, A5 to A8, A9 to A12, etc.) starting with a catalog number and ending with an arrow head (see plate map). This plate design will result in four polymer (P) to DNA (D) mass ratios (P:D=200:1, 100:1, 50:1 and 25:1) if the recommended amount of plasmid is added. Once the polymers for which the best transfections are identified, refer the catalog numbers for individuals polymer ordering.

Figure 2. *Leopard*™ Transfection Array v1.0 Guide to Polymer Identification and Ordering

Plate Map:

	1	2	3	4	5	6	7	8	9	10	11	12
	*200:1	100:1	50:1	25:1	200:1	100:1	50:1	25:1	200:1	100:1	50:1	25:1
A	[Y]CLI4376				CLI4384				CLI4392			
B	CLI4377				CLI4385				CLI4393			
C	CLI4378				CLI4386				CLI4394			
D	CLI4379				CLI4387				CLI4395			
E	CLI4380				CLI4388				CLI4396			
F	CLI4381				CLI4389				CLI4397			
G	CLI4382				CLI4390				CLI4398			
H	CLI4383				CLI4391				empty			

* The polymer to DNA mass ratio obtained if recommended amount of polymer and DNA are used.
[Y] CLI4376 to CLI4398 are catalog numbers for individual polymers.

Example:
A technician treats a 96-well plate of COS-1 cells with the *Leopard* Transfection Array v1.0, uses the β-galactosidase reporter plasmid supplied with the kit, and assays the cells for β-galactosidase expression using the All-in-One™ Mammalian β-Galactosidase Assay Reagent from Pierce. Wells B2, B3 and B4 show a light yellow color, wells E5, E6, E7 and E8 show an intense yellow color, increasing from left to right, and wells D9 and D10 show intense yellow color, decreasing from left to right. The technician would then use the Plate Map above to identify the catalog numbers to order polymers CLI4380 and CLI4395. The individual polymers will be provided at a single concentration (20mg/ml in 100% DMSO). If the best result was obtained with a 200:1 P:D ratio, undiluted polymer would be used in follow-up experiments. If 100:1 P:D was determined to be optimum, then the provided polymer would be diluted 1/2 in DMSO before use. If 50:1 P:D was determined to be optimum, then the provided polymer would be diluted 1/4 in DMSO before use. If 25:1 P:D was determined to be optimum, then the provided polymer would be diluted 1/8 in DMSO before use. To harness the full power of the system, the technician could take the information from the experiment regarding the specific polymer, as well as the P:D ratios, that gave the best results, and further optimize the transfection process by examining amount of plasmid used, transfection time, and P:D ratios not covered in the plate.

In addition, once effective polymer(s) have been identified using a reporter vector, it is recommended that further optimization is performed using specific cloned gene(s) of interest. The appropriate assay used to detect the transfected gene will depend on the specific gene. This may be an enzymatic, immunohistochemical, RT-PCR, *in situ* hybridization, or morphologic assay depending on the known properties of the transfected gene. The details of the assay to be used should be defined prior to setting up experiments as would be required for any transfection experiment. It is important to include negative controls for comparison of results. These should include transfection with plasmid only as well as a transfection with no added plasmid. Four wells on the plate (H9 through H12) are left empty so that you can include appropriate controls and/or other reagents of your choice during your screening for comparison.

V. References

1. Anderson D.G., A. Akinc, N. Hossain, & R. Langer. 2005. Structure/property studies of polymeric gene delivery using a library of poly(beta-amino esters). *Mol Ther*. **11**:426-34.

2. Akinc, A.D. Anderson, D.M. Lynn, & R. Langer. 2003. Synthesis of Poly(β-amino ester)s Optimized for Highly Effective Gene Delivery. *Bioconjugate Chem.* **14**:979-988.

3. Akinc, A. D.M. Lynn, D.G. Anderson, & R. Langer. 2003. Parallel Synthesis and Biophysical Characterization of a Degradable Polymer Library for Gene Delivery. J. Am. Chem. Soc. 125:5316-5323.

VI. Appendix

a. Vendor Information for Supplementary Materials

β-gal reporter vector, cat. # RHS3708

β-Gal reporter vector
Open Biosystems, Inc.
www.openbiosystems.com

Reduced-serum medium for transfection, cat. # 11058021

Opti-MEM®
Invitrogen Corporation
www.invitrogen.com

β-galactosidase assay, cat. # 75705

All-in-One™ Mammalian β-Galactosidase Assay Reagent
Pierce
www.piercenet.com

Contact and Support

For answers to questions or to suggest improvements or share new applications, feel free to call us at: 888-412-2225, email to info@openbiosystems.com, or visit our website at www.openbiosystems.com