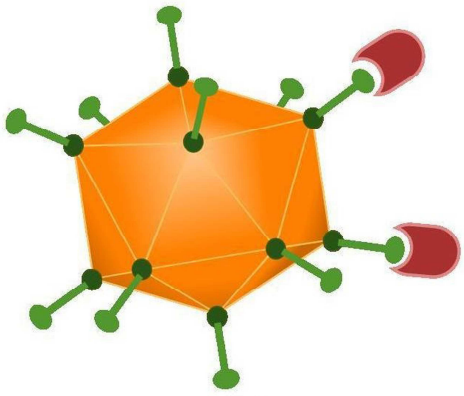
AdenoBlast

INSTRUCTION MANUAL

Adenovirus Transduction Enhancer



AdenoBlast

OZBIOSCIENCES
The art of delivery systems
www.ozbiosciences.com

AdenoBlast

Instruction Manual

AdenoBlast is the ideal Adenoviral Transduction Enchancer**.**

List of AdenoBlast kits

Catalog Number	Description	Volume (µL)	Number of transduction at 1.10^7 IU
AB00125	AdenoBlast 50 transductions	125 µL	50
AB03125	AdenoBlast 150 transductions	3 x 125 µL	150

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (order@ozbiosciences.com). For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprises
13288 Marseille cedex 09 - France
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

1. Technology

1.1. Description

AdenoBlast is the ideal reagent developed to enhance adenoviral infection and transduction in any type of cells, adherent or in suspension, primary or cell lines. The use of adenoviral transduction requires the Coxsackie Adenovirus Receptor (CAR) for its initiation. Many cells lack or express only low amounts of CAR thus making adenovirus-mediated transduction limited. **AdenoBlast** is based on an adenovirus binding peptide that assists transduction by coupling the adenoviral particles to the cell membrane in a CAR independent manner. Therefore adenoviral transduction is now possible even in non-permissive cells. **AdenoBlast** is non-toxic and totally compatible with cell viability.

NOTE: AdenoBlast is especially recommended if high multiplicities of infection (MOI) are used.

AdenoBlast adenoviral transduction reagent principal advantages:

- Enhances infection and transduction efficiency of adenovirus
- Ideal for permissive and non-permissive cells
- Non-toxic (potential for *in vivo* applications)
- Compatible with cell lines and primary cells,
- Allows using reduced amounts of Adenovirus (low MOI), reducing the cost

1.2. Kit Contents, Stability and Storage

Contents

Kits content varies according to their size:
AdenoBlast 50 transductions :

- 1 tube containing 125 µL of AdenoBlast good for up to 50 transductions at 1.10^7 IU.

AdenoBlast 150 transductions :

- 3x1 tube containing 125 µL of AdenoBlast good for up to 150 transductions at 1.10^7 IU.

Storage and Shipping

Storage: Upon reception and for long-term use, store the AdenoBlast transfection reagent at 4°C.

Shipping condition: Room Temperature

2. Applications

AdenoBlast is based on an adenovirus binding peptide and has been specifically developed for enhancing adenoviral transduction in any type of cell, adherent or in suspension, primary or cell lines. **AdenoBlast** functions as an alternative receptor for the CAR binding unit increasing affinity to the cell surface (Figure 1).

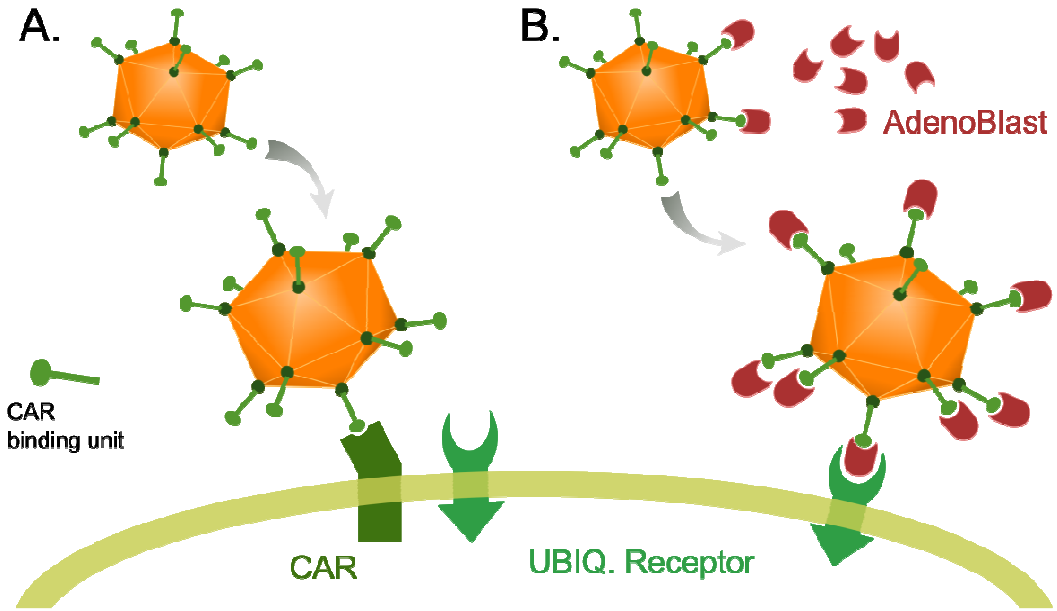


Figure 1. Adenoviruses bind CAR receptor at the surface of permissive cells through their CAR binding units (A). AdenoBlast bridges adenoviral particles to the cell membrane and enhances the CAR binding unit affinity to an ubiquitous receptor on the cell's surface (B). Transduction occurs in a CAR-independent manner and gene expression is enhanced by 20 to 50 times.

3. General Protocol

3.1. General Information / Important Guidelines

AdenoBlast efficiency is highly related to the MOI used: depending on the cell line, several MOI have to be tested. Refer to Table 1 below for the recommended volume of AdenoBlast depending on the MOI used and the well format.

Table 1: Cell number, volume for transduction and AdenoBlast volume per well in 48-, 24- and 96-well plates.

Format	Cell number/well	Volume for transduction	MOI 40	MOI 200	MOI 500	MOI 1000
48 wells	25.000	125 µL	0.25 µL	1.25 µL	3.13 µL	6.25 µL
24 wells	50.000	250 µL	0.5 µL	2.5 µL	6.25 µL	12.5 µL
12 wells	100.000	500 µL	1.0 µL	5.0 µL	12.5 µL	25.0 µL

3.2. Cells Preparation

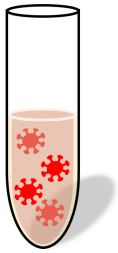
Cell culture prior to transduction: prepare the cells according to Table 1 above the day before transduction. It is recommended to plate the cells in classical culture medium. Cells should be 50-70 % confluent at the time of transfection.

3.3. Standard Protocol

Use the following protocol to find the ideal conditions for AdenoBlast in 24-well plate. If the adenoviral transduction/infection conditions are unknown, we recommend starting with several MOI using an adenoviral vector encoding for a fluorescent protein.

Note: We suggest to use **0.5 to 12.5 µL of AdenoBlast** using **MOI of 40 to 1000.**

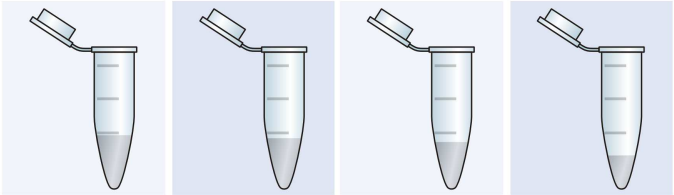
1. VIRUS PREPARATION



Dilute virus into complete culture medium sufficient for 4 samples.

We recommend preparing an adenoviral solution containing 5.10^8 viral particles/mL.

2. PREPARE 4 TUBES CONTAINING MEDIUM (250 µL final)



245.5 µL	227.5 µL	193.75 µL	137.5 µL
Tube 1	Tube 2	Tube 3	Tube 4

3. ADD ADENOBLAST TO EACH TUBE

	Tube 1	Tube 2	Tube 3	Tube 4
AdenoBlast	0.5 µL	2.5 µL	6.25 µL	12.5 µL

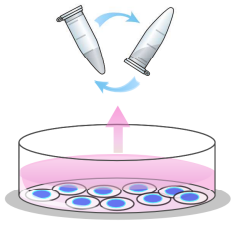
4. ADD ADENOVIRAL PARTICLES CORRESPONDING TO MOI

	Tube 1	Tube 2	Tube 3	Tube 4
MOI	40	200	500	1000
Adenoviral suspension	4 µL	20 µL	50 µL	100 µL

5. MIX VIALS BY INVERTING

Do not vortex or centrifuge

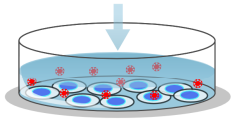
incubate 30min at RT using a shaker (400 rpm)



6. ASPIRATE MEDIUM FROM CELLS

7. ADD ADENOVIRUS + ADENOBLAST

Incubate the cells 4 h under standard culture conditions



8. CHANGE MEDIUM

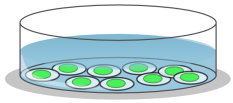
Remove medium from the cells

wash twice in PBS and add pre-warmed culture medium

9. INCUBATE CELLS 24 TO 96 h.

Incubate the cells under standard culture conditions

We recommend performing assay from 24 to 96 h.



4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

4.1 Quality Controls

To assure the performance of each lot of AdenoBlast reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

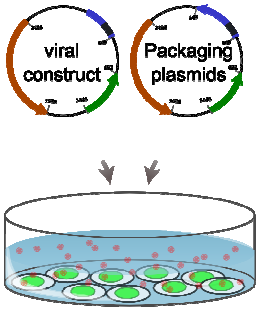
Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of contamination shall be obtained for 15 days.
Biological Activity	Transduction efficiency on NIH-3T3 cells. Every lot shall have an acceptance specification of > 90% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transduction efficiency	1- **MOI used is low.** Raise the adenoviral particles amount: Up to MOI 1000 can be used. 2- **Infectious viral titer is low.** Check viral titer via transduction of 293A cells with serial dilutions of adenovirus. 3- **Cells are difficult to transduce.** Raise both AdenoBlast and MOI. 4- **No effect of AdenoBlast is observed.** Concentrations of AdenoBlast is too low, use more AdenoBlast with the same MOI. 5- **Cell Density is too high on the day of transduction.** Lower the cell number. Ideally cells should be at 50-70% visual confluence on the day of transduction.
Low viability	1- **MOI is too high.** Lower the adenoviral dose while slightly raising the AdenoBlast volume. 2- **AdenoBlast concentration is too high.** Decrease concentration of AdenoBlast. 3- **Cells are sensitive to adenovirus/AdenoBlast treatement.** Replace medium with fresh medium 4 to 6 h after transduction. 4- **Too strong side effects.** Reduce MOI. 5- **Cells are sensitive to low amounts of media.** Use more media during transduction.

5. Related Products

Viral production

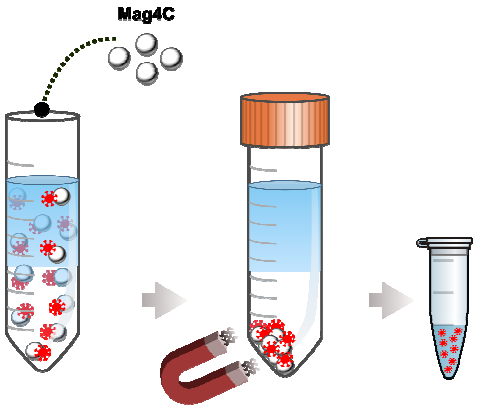


Calcium Phosphate transfection Kit

DreamFect Gold

- high transfection efficiency
- high compaction level
- biodegradable

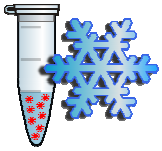
Capture / Concentration



Mag4C Kits
- Mag4C-Lv
- Mag4C-Ad

- concentrate virus
- raise viral dose
- isolate virus from inhibitor
- conserve the viral particles

Conservation

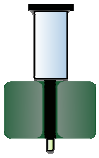


Mag4C conservation buffer

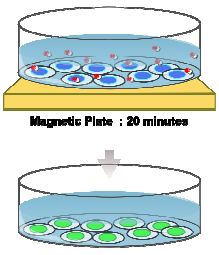
- Adenoviral particles
- Retro/lentiviral particles

Infection Enhancement

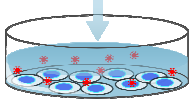
cell sorting column



Magnetofection



BioChemicals



i-MICST: ViroMICST

Magnetic

ViroMag
ViroMag R/L
AdenoMag

- viral concetration onto the cell surface
- raise transduction efficiency
- synchronize infection

BioChemicals

LentiBlast
AdenoBlast

- Enhance transduction
- non toxic
- Low MOI

6. Purchaser Notification

Limited License

The purchase of the AdenoBlast reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents) for the sole purpose of in-house research only, provided that no license, right or permission is granted hereunder to a non-academic, for-profit or commercial Licensee to use the AdenoBlast reagent for *ex vivo* gene therapy for hemoglobinopathies. The license does not include the use for any commercial or development purpose, including but not limited to any use for a) manufacturing, production, quality control, b) providing services, information or data, c) therapeutic, diagnostic, vaccine or prophylactic purposes or d) any applications which require regulatory approval as well as e) any clinical activities *in vivo* or *ex vivo*. The licensed use is limited to transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

To inquire about separate licenses for the express purpose of non-research use or applications of the AdenoBlast reagent, or to obtain authorization to transfer or use the enclosed material, please contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all AdenoBlast reagent material and documentation to OZ Biosciences, or by destroying all AdenoBlast reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a AdenoBlast reagent kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of AdenoBlast reagent research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The AdenoBlast reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
163, avenue de Luminy, zone entreprise, case 922
13288 Marseille Cedex 9, France
Tel: +33 (0)4.86.94.85.16
Fax: +33 (0)4. 86.94.85.15
E-mail: business@ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

www.ozbiosciences.com

Follow us!