Viral Applications

AAVBlast

Transduction reagent

Enhance AAV infection and tranduction efficiency

Protocol

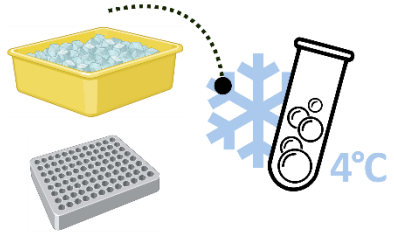
We Bring The World Of Biotechnology To You
BOCA SCIENTIFIC

OZBIOSCIENCES
The art of delivery systems

AAVBlast Quick Protocol

1. AAVBlast

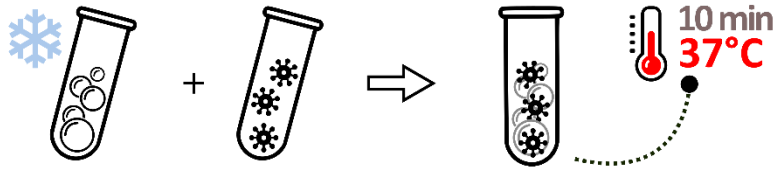
Thaw AAVBlast at 4°C and keep it in a melting ice bath or cooling rack



keep AAVBlast cool!

2. Preparation of the complexes

Add 0.5 µL-2 µL of AAV Blast to viral suspention in 10 to 15 µL medium without supplement and place them at 37°C immediately for 10 min.



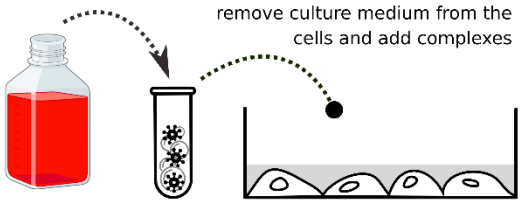
AAVBlast
0.5 to 2 µL

AAV solution
(10 - 15 µL)

Place the complexes at 37°C for 10 min

4. Cell preparation

Add medium without FBS or supplement



QS 250 µL

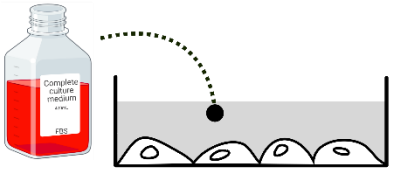
Add complexes to the cells

5. Incubation

Incubate for 2 hours

6. Cell preparation

Add complete culture medium that may contain 2X more FCS



Add complete culture medium and incubate until evaluation of the experiment

1) Plate the cells **24 h** before the experiment to reach **80-90%** confluence on the day of transduction
2) Thaw AAVBlast at **4°C** and keep it at **4°C** in a melting ice bath or cooling rack to prevent gelation and ensure a correct pipetting
3) Prepare AAV working solution in **10** to **15 µL** culture medium w/o supplement

NOTE: to ensure the best complexation efficiency, use the lowest possible volume (max 1/10th of the total culture medium).

4) Add **0.5** to **2 µL** AAVBlast to viral suspension
5) Immediately incubate at **37°C** for **10 min**
6) Add culture medium w/o supplement to AAV/AAVBlast complexes – **QS 250µL**
7) Remove culture medium from cells
8) Add complexes to the cells
9) Incubate 2h under standard culture conditions
10) Add **250 µL** complete culture medium*****.

*****in case of sensitive cells, prepare complete culture medium containing two times more serum in order to counter-balance its absence in the transfection medium.

Optionally, a medium wash can be performed after the 2-hours incubation procedure: remove transduction medium and add 500 µL of complete culture medium.

IMPORTANT NOTES – Before you begin

- ✓ During the experiment, keep AAVBlast at 4°C in a melting ice bath or cooling rack to prevent gelation and ensure a correct pipetting.
  We recommend using a cooling rack to decrease risk of contamination.

- ✓ Do not change the transduction conditions already settled: simply add the AAVBlast to your already established protocol.

- ✓ It normally takes at least 48h to visualize transduction that can be followed up to 6 days. Generally, no more increase is observed after 5 days.

- ✓ It is mandatory to use medium without any supplement for the preparation of complexes. Culture mediums such as DMEM (with or w/o phenol red), RPMI (with or w/o phenol red), DMEM-F12, alpha-MEM, EMEM or OptiMEM are recommended.

- ✓ Do not use AAVBlast with another viral enhancer or adjuvant.

- ✓ If the transduction conditions are unknown, we recommend starting with MOI ranging from 1000gc (genome copies) to 3000 gc per cell using AAV encoding for a fluorescent protein.

- ✓ In case of low efficiency using the standard protocol, transduction efficiency can be raised:

  a. **by reverse transduction:** depending on the cell type and the virus serotype, a reverse transduction procedure can be performed. Please see below for more details.
  b. **by optimization:** Variations in MOI, volumes of AAVBlast can lead to higher transduction efficiency.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	AAVB250: 250 µL of AAVBlast Reagent
Shipping conditions	Room Temperature
Storage conditions	Store the AAVBlast Reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	AAVBlast is ideal to enhance AAV infection and transduction in any type of cells: adherent or in suspension, primary or cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

Cell culture prior to transduction: the day before transduction prepare the cells according to the table below. Cells should be 80-90 % confluent at the time of transduction (see the suggested cell number in the Table 1).

Tissue Culture Dish	Cell Number
96 wells	8 – 15 x 1.10^3
24 wells	4 – 8 x 1.10^4
6 wells	2 – 4 x 1.10^5

Table 1: Suggested cell number for AAV transduction (per well)

IMPORTANT NOTE

For hard-to-transduce or non-permissive cells, and depending on serotype, prepare the cells the day of transduction and refer to reverse-transduction procedure below.

2. Standard Protocol

Use the quick protocol above to find the ideal conditions for AAVBlast in 24-well plate.
If the AAV transduction/infection conditions are unknown, starting with MOI ranging from 1000 gc (genome copies) to 30000 gc per cell using an AAV encoding for a fluorescent protein.

NOTE: We suggest using 0.5, 1 µL and 2 µL of AAVBlast per condition.

3. Reverse-transduction Protocol

Reverse transduction, alternatively referred to as substrate-mediated gene delivery, involves the initial coating of viral vectors onto a surface.

It may be a good alternative to classic transduction depending on cell type or virus serotype.

1) Follow steps 2 to 5 of the quick protocol
2) Add AAV/AAVBlast mixes to the plate
3) Store the plates at 4°C overnight
4) On the day of transduction, carefully aspirate the virus solution from the plate
5) Add the cells to the plate in their complete culture medium
6) Optionally, cells can be added in medium w/o supplement for 2H before serum supplementation

Optimization Protocol

Optimizing AAVBlast volumes

To find the ideal transduction conditions using AAVBlast, we recommend optimizing volumes of AAVBlast with a fixed MOI (refer to Table 2).

Tissue culture dish format	1:25	1:50	1:125	1:500	1:1000
24-well plate	10 µL	5 µL	2 µL	1 µL	0.5 µL

Table 2: Recommended volumes of AAVBlast for optimization

NOTES

Additional products for Viral Transduction Enhancement

- **LentiBlast Premium** for enhancing lentiviral infection and transduction efficiency
- **ViroMag** for enhancing viral transduction efficiency (suitable for all viruses)
- **ViroMag R/L** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Capture and Concentration

- **Mag4C-LV** for Lentiviruses
- **Mag4C-AD** for Adenoviruses

Purchaser Notification

Limited License
The purchase of the AAVBlast 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 AAVBlast 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 AAVBlast 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 AAVBlast reagent material and documentation to OZ Biosciences, or by destroying all AAVBlast reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a AAVBlast 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 AAVBlast 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 AAVBlast 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.

We Bring The World Of Biotechnology To You
BOCA SCIENTIFIC
www.bocascientific.com
(781) 686-1631
info@bocascientific.com

OZBIOSCIENCES
The art of delivery systems

Rev. 11/2023