AAVBLAST Results

1. Description.

AAVBLAST, is a chemical AAV transduction enhancer, very effective to promote viral-mediated genetic modification. AAVBlast improves transduction of various AAV serotypes in a wide range of cell types from classic cell lines to primary cells or mesenchymal stem cells. Its patented formulation and unique thermoresponsive gelling properties, ensure the protection of viral particles and allow an increase in transduction efficiency. Non-toxic, the new AAVBlast AAV transduction enhancer is also compatible with *in vivo* experiments.

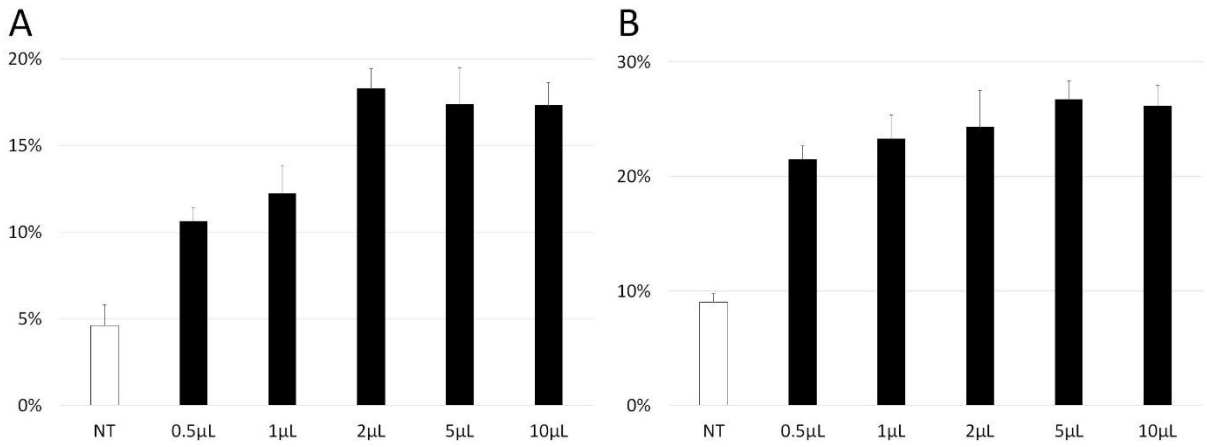
2. Storage and shipping condition.

Storage: -20°C upon reception and for long-term use.
Stability: 1 year
Shipping condition: The reagent is shipped at RT

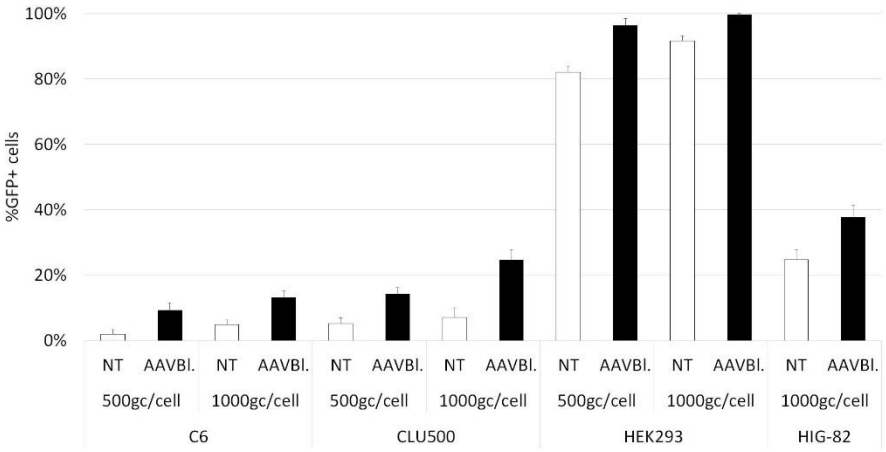
AAVBLAST enhances AAV transduction efficiency in cell lines

AAVBLAST allows enhancing transduction of AAV2 and AAV6 in a dose-dependent manner.

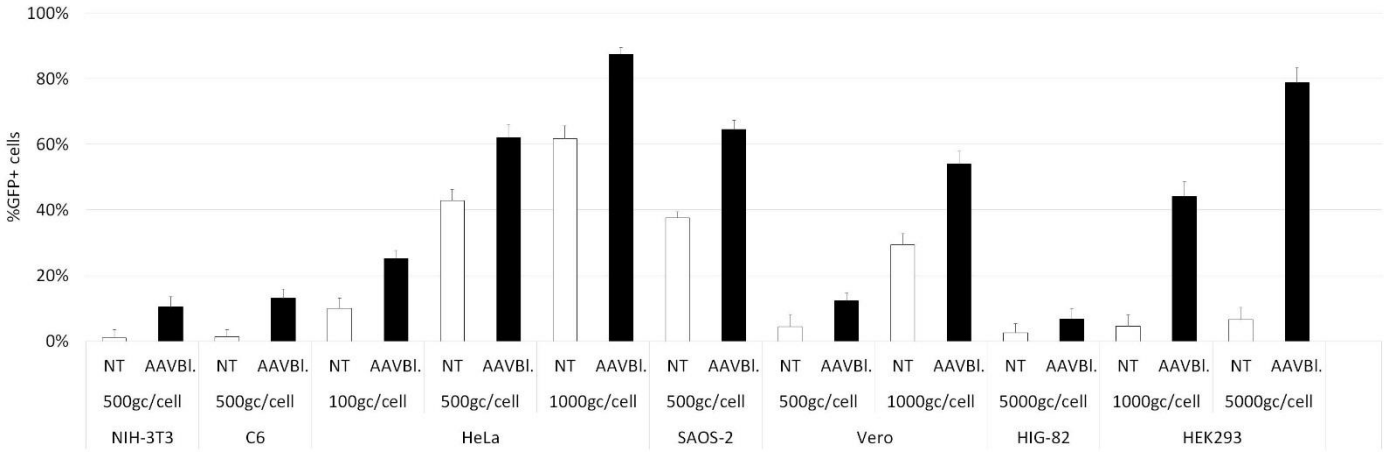
Complexes of AAV2 and AAV6 serotypes viral particles encoding for GFP and AAVBlast were formed by a 10min incubation time at 37°C according to the protocol before addition to cells. Medium was complemented with serum 2h later and results were analyzed after 72h of incubation. The percentage of GFP+ cells was determined by flow cytometry.



NIH-3T3 cells were transduced with AAV2 (A) or AAV6 (B) at respectively MOI of 1000gc/cell and 5000gc/cell in presence or not of ranging doses of AAVBlast. % of GFP+ cells was determined 72h after transduction by flow cytometry.



C6, CLU-500, HEK293 and HIG-82 cell lines were transduced with AAV2 at one or two MOIs in presence or not of AAVBlast according to the protocol. % of GFP+ cells was determined 72h after transduction by flow cytometry.



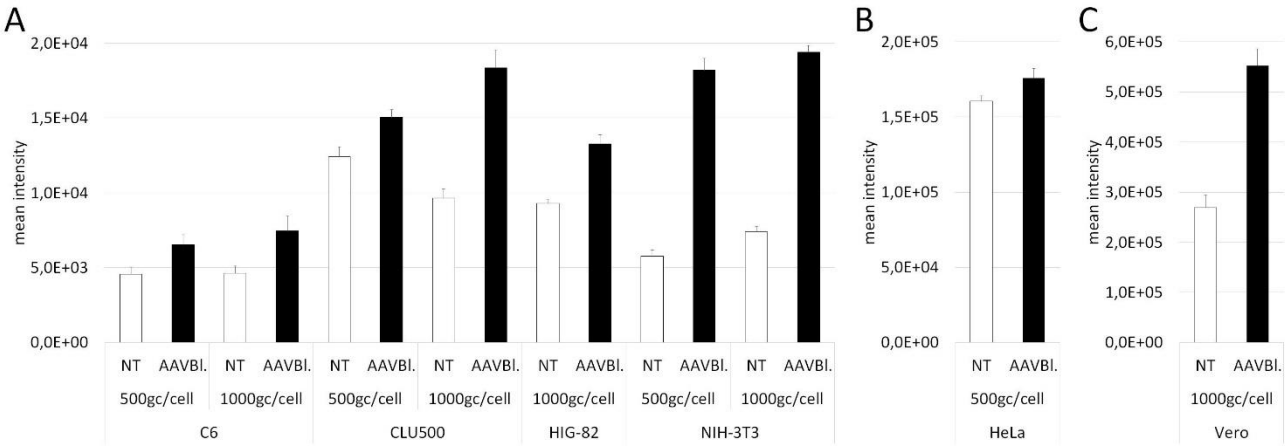
NIH-3T3, C6, HeLa, SAOS-2, Vero, HIG-82 and HEK293 cell lines were transduced with AAV6 at one, two or three MOIs in presence or not of AAVBlast according to the protocol. % of GFP+ cells was determined 72h after transduction by flow cytometry.

These results demonstrate the capacity of AAVBlast to efficiently increase transduction efficiency of AAV2 and AAV6 in a wide range of cell types.

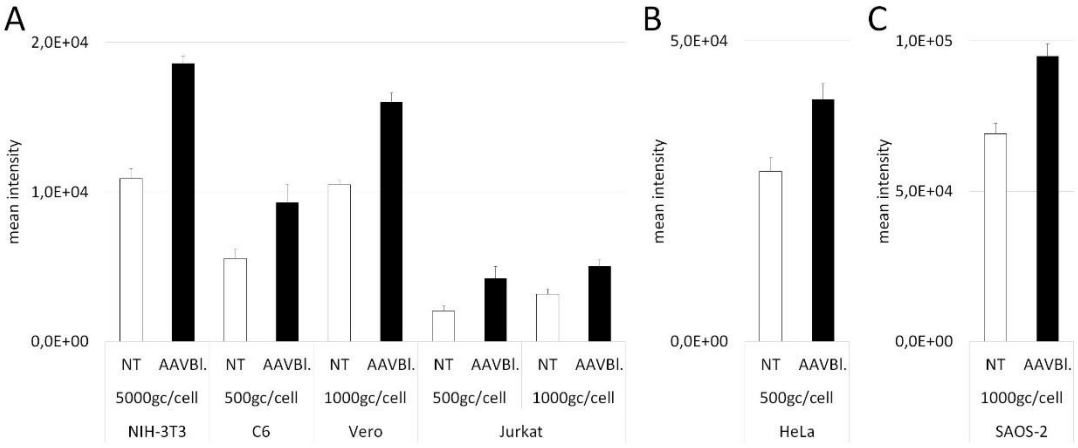
AAVBLAST allows to enhance transgene expression following transduction

AAVBLAST increases the % of transduced cells as well as transgene expression.

Complexes of AAV2 and AAV6 serotypes viral particles encoding for GFP, and AAVBlast were formed by a 10min incubation time at 37°C according to the protocol before addition to cells. Medium was complemented with serum 2h later and results were analyzed after 72h of incubation. The percentage of GFP+ cells was determined by flow cytometry.



C6, CLU-500, HIG-82 and NIH-3T3 cell lines (A), HeLa cells (B) and Vero cells (C) were transduced with AAV2 at one or two MOIs in presence or not of AAVBlast according to the protocol. Fluorescence intensity of GFP+ cells was determined 72h after transduction by cytofluorometry.



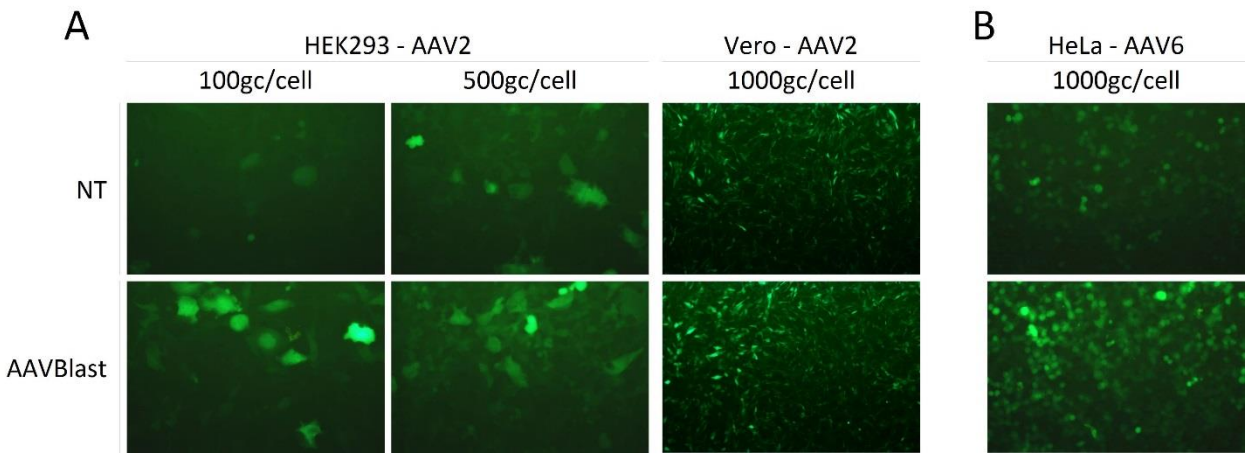
NIH-3T3, C6, Vero and Jurkat cell lines (A), HeLa cells (B) and SAOS-2 cells (C) were transduced with AAV6 at one or two MOIs in presence or not of AAVBlast according to the protocol. Fluorescence intensity of GFP+ cells was determined 72h after transduction by cytofluorometry.

These results demonstrate the capacity of AAVBlast to efficiently increase transgene expression of AAV2 and AAV6 in a wide range of transduced cell types.

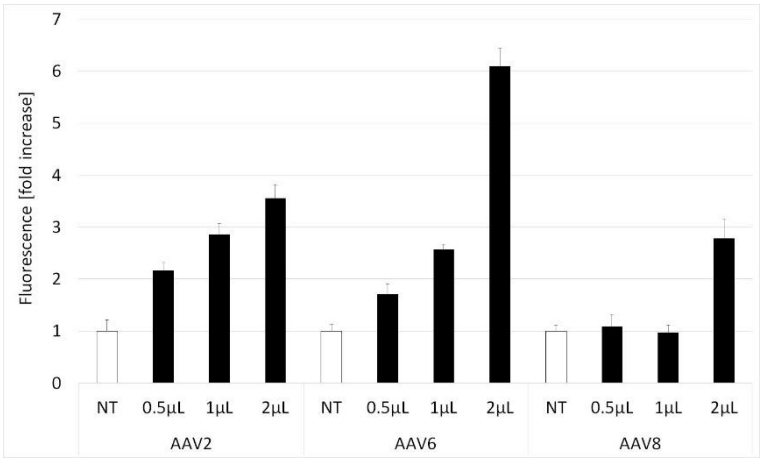
AAVBlast and AAV serotypes for cell lines and Mesenchymal Stem Cells

AAVBLAST allows increasing transduction of various AAV serotypes in cell lines and mesenchymal Stem Cells.

AAV2, AAV6 and AAV8 serotypes viral particles encoding for GFP were complexed with AAVBlast by a 10min incubation time at 37°C according to the protocol before addition to cell lines or ovine Mesenchymal stem cells (oMSC). Medium was complemented with serum 2h later and results were analyzed after 72h of incubation. Transduction efficiency of cell lines was monitored by fluorescence microscopy and the percentage of GFP+ oMSC was determined by flow cytometry.



HEK293 & Vero cell (A) and HeLa cells (B) were transduced respectively with AAV serotype 2 and serotype 6 in presence or not of AAVBlast according to the protocol. 72h after transduction, GFP expression was monitored under fluorescent microscopy.



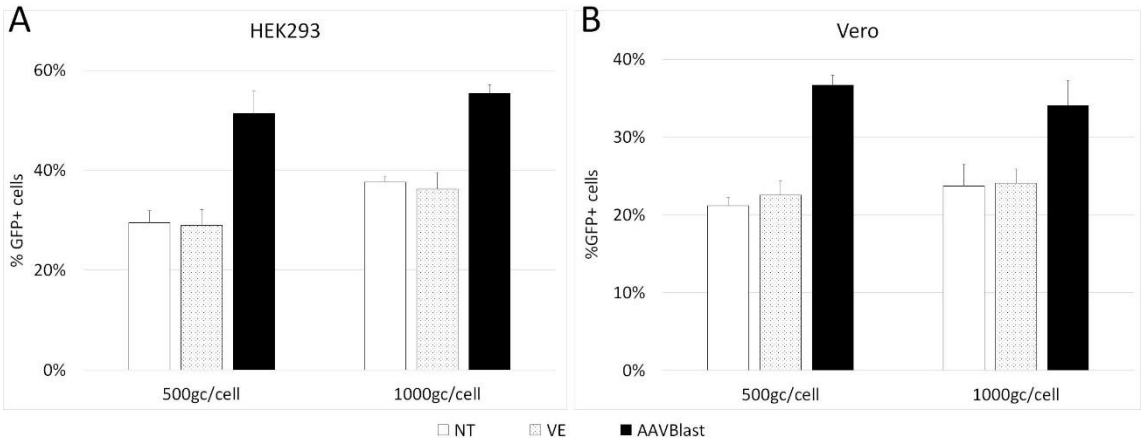
Ovine mesenchymal stem cells were transduced with AAV serotype 2, -6 or -8 encoding for GFP in presence or not of ranging doses of AAVBlast. Fold increase in fluorescence intensity of GFP+ cells was determined 72h after transduction by cytofluorometry.

Results show that AAVBlast is easily adaptable to various AAV serotypes.

AAVBLAST comparison to competitor.

AAVBLAST increases the transduction of AAV viral particles compared to competitor

AAV2 viral particles encoding for GFP were complexed to a commercial Viral Enhancer (VE) or AAVBlast according to their respective protocols. Medium was complemented with serum 2h later for AAVBlast and results were analyzed after 72h of incubation. The percentage of GFP+ cells was determined by flow cytometry.



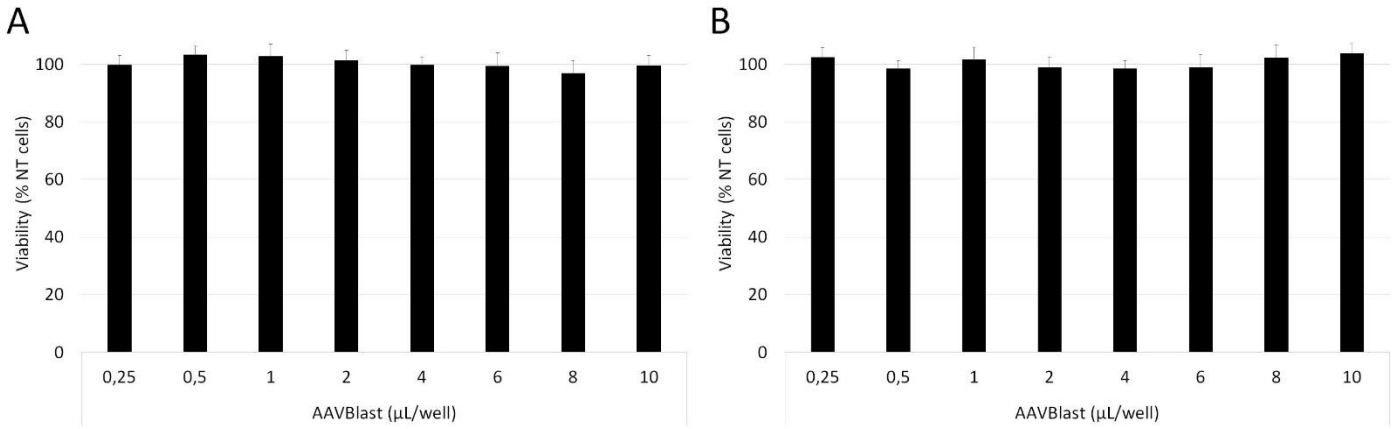
HEK293 (A) and Vero cells (B) were transduced with AAV2 at two MOI in presence or not of commercial viral enhancer (VE) or AAVBlast according to their respective protocol. % of GFP+ cells was determined 72h after transduction by flow cytometry.

Results demonstrate that AAVBlast outperforms commercial competitor for AAV2 transduction enhancement in HEK293 and Vero cell lines.

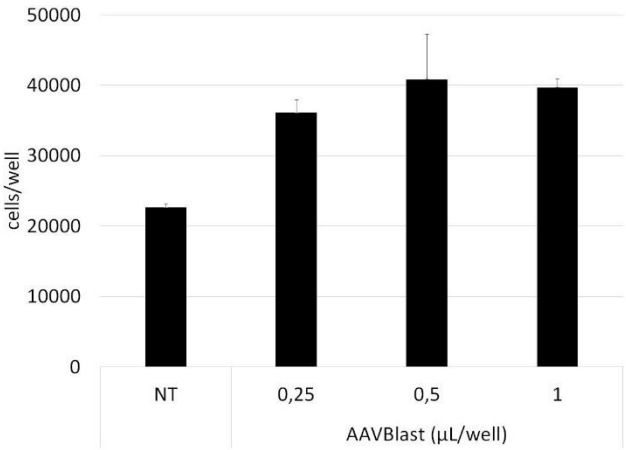
AAVBlast, viability and proliferative state

AAVBlast does not have any impact on cell viability and allows mesenchymal stem cells to proliferate.

Vero and HeLa cell lines were cultivated in 96-well plates and ovine mesenchymal stem cells were seeded in 24-well plates. Both cell lines and primary oMSC were transduced with AAV2 incubated with ranging doses of AAVBlast. 72 hours later, determination of their viability index was realized using different cellular assay kits, WST-8 cell proliferation assay kit, OZBlue resazurin cell viability kit or XTT assay kit.



Vero cells and HeLa cell line were transduced with AAV2 in presence of ranging doses of AAVBlast. 72H after, viability was determined by WST-8 cell proliferation kit (OZBiosciences Ref #WS1000) on Vero cells (A) or by OZBlue cell viability Kit (OZBiosciences #BL00100) on HeLa cells.



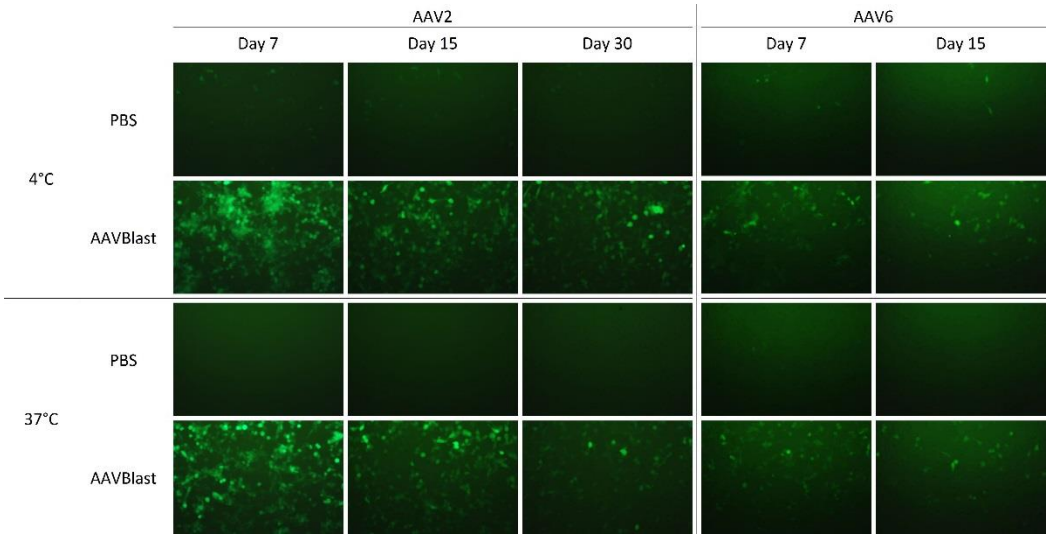
Cell survival of Ovine Mesenchymal Stem Cells (oMSC) transduced with AAV2 in presence of ranging doses of AAVBlast. 72h after, viability was determined by XTT assay Kit.

Results demonstrate that AAVBlast not only does not impair viability in cell lines but also induces a positive proliferative rate in ovine mesenchymal stem cells.

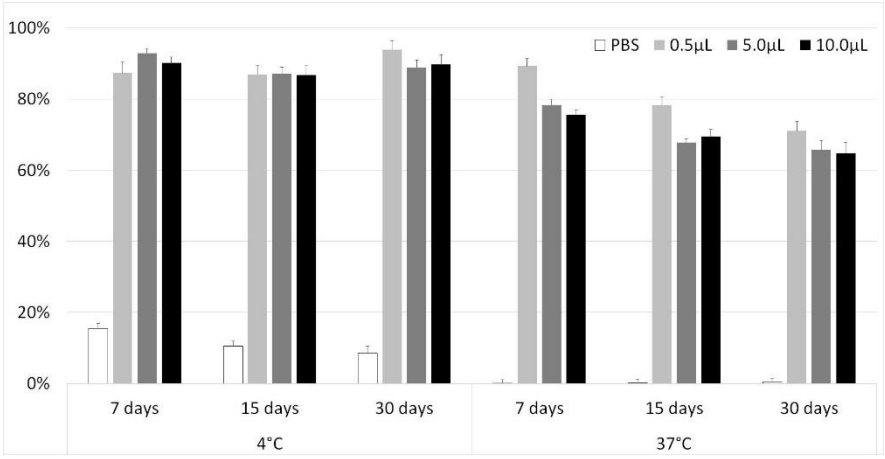
AAVBlast and stabilization of AAV particle for a better conservation

AAVBlast allows to stabilize the AAV particles and preserves their efficiency even when stored at 37°C.

AAV2 and AAV6 viral particles were mixed with PBS or AAVBlast and incubated at 37°C as recommended by the protocol. After 10min, complexes were stored either at 4°C and 37°C over a month. HeLa cells were transduced with the complexes 7, 15 or 30 days after complex preparation and infection was monitored by fluorescence microscopy or flow cytometry 72h later.



AAV2 or AAV6 were mixed with PBS or 0.5 µL AAVBlast and stored at 4 or 37°C. After 7, 15 and 30 days, HeLa cells were transduced with viral suspensions and fluorescence was observed 72h after under microscope.



AAV2 were mixed with PBS or ranging doses of AAVBlast (0, 0.5, 5 & 10µL) and stored at 4 or 37°C. After 7, 15 and 30 days, HeLa cells were transduced with viral suspensions and % of GFP+ cells was determined by flow cytometry after 72h.

Results demonstrate that AAVBlast allows to protect and stabilize AAV viral particles. To be noted, the increase in AAVBlast volume does not enhance protection in this model of experiment.