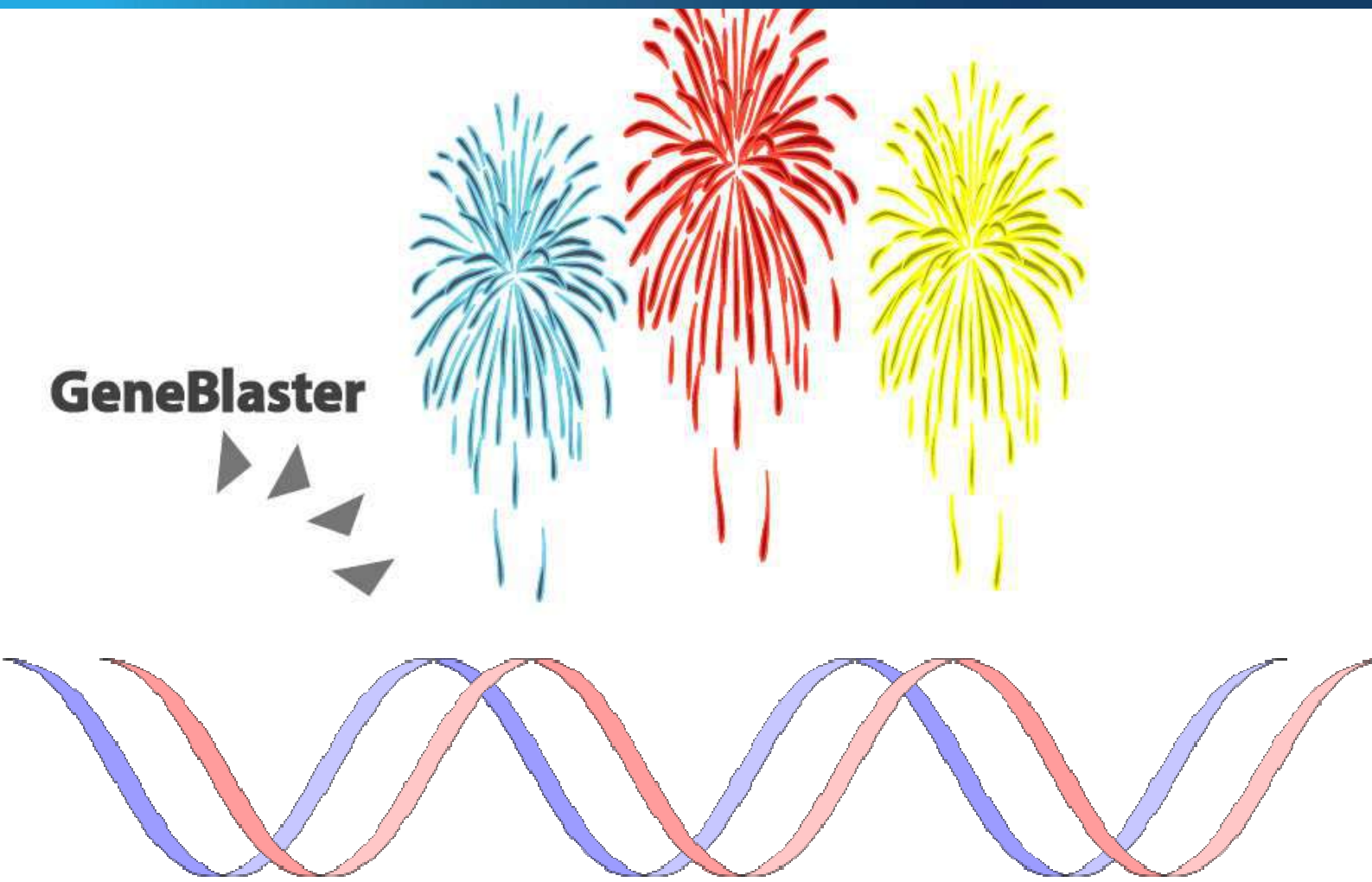
GeneBlaster™

INSTRUCTION MANUAL



OZBIOSCIENCES
The art of delivery systems
www.ozbiosciences.com

We Bring The World Of Biotechnology To You
BOCA SCIENTIFIC

GeneBlaster Kit

Instruction Manual

GeneBlaster Kits are new formulations of chemicals that significantly enhance and prolong the gene expression level obtained by *in vitro* transfection.

List of GeneBlaster Kits

Catalog Number	Description	Volume
GB20010	GeneBlaster Selection Kit [1]	4 x 1.5 mL
GB20011	GeneBlaster Ruby	3 x 1.5 mL
GB20012	GeneBlaster Sapphire	3 x 1.5 mL
GB20013	GeneBlaster Topaz	3 x 1.5 mL
GB20014	GeneBlaster Emerald	3 x 1.5 mL

[1] Contain 1 vial of each GeneBlaster reagent (Ruby, Sapphire,Topaz & Emerald)

www.bocascientific.com • (781) 686-1631 • info@bocascientific.com

1. Technology

1.1. Description

Congratulations on your purchase of the ***GeneBlaster*** Kit!

The ***GeneBlaster*** Kits are new formulations of chemical mixtures that significantly improve the gene expression level obtained with viral and non-viral gene delivery systems such as Magnetofection™, DreamFect™, DreamFect™ Gold, VeroFect and EcoTransfect reagents. Furthermore, they can be used with all commercially available transfection reagents. Since the application of the GeneBlaster Kits is cell type and promoter dependent three special formulations have been developed accordingly. They are extremely easy to use; simply add the appropriate GeneBlaster reagent to your culture medium and you get higher and longer levels of transgene expression.

- Rapid and easy to use
- Highest gene expression in many cells
- Prolong in vitro gene expression
- Excellent enhancement with transfection reagents such as Magnetofection™ and EcoTransfect™
- Effective for both transient and stable transfection
- Economical

1.2. Available Kits

OZ Biosciences offers four types of ready-to-use GeneBlaster Kits:

1. **GeneBlaster Ruby** is a mixture of chemicals that has been developed intentionally for adherent cells. The dimension of the response is also cell type depend.
2. **GeneBlaster Sapphire** is another combination of chemicals developed for adherent cells. The response extend is also cell type depend and complement well the GeneBlaster Ruby.
3. **GeneBlaster Topaz** is a mixture of chemicals that has been developed purposely for suspension cells especially hematopoietic cell lines. However, other cell types appear to respond also very well.
4. **GeneBlaster Emerald** is a new formulation of additives that significantly enhance and prolong the gene expression level obtained by *in vitro* transfection in primary neurons.
5. **GeneBlaster Selection Kit**. This kit is a convenient assortment of the four GeneBlaster reagents that permit to cover a large number of suspension and adherent cells.

1.3. Kit Contents

Each vial of the GeneBlaster Reagent (1.5 ml) is provided at a 100 X concentration and is sufficient for 150 transfections using 1 ml transfection volume. Each Kit contains three vials of reagents and allows performing at least 450 assays.

Kit contents

Description	Vials	Volume	Tube color
GeneBlaster Selection Kit [1]	3	4 x 1.5 mL [1]	1 red, 1 blue, 1 yellow,1 green
GeneBlaster Emerald	3	3 x 1.5 ml	3 green
GeneBlaster Ruby	3	3 x 1.5 mL	3 red
GeneBlaster Sapphire	3	3 x 1.5 mL	3 blue
GeneBlaster Topaz	3	3 x 1.5 mL	3 yellow

[1] Contains 1 vial of each GeneBlaster reagent (Ruby, Sapphire,Topaz & Emerald)

Stability and Storage

Storage — Upon receipt and for long-term use, store all reagent tubes at -20°C or + 4°C. GeneBlaster Kits are stable for at least 6 months at + 4°C or -20°C.

Shipping condition — The GeneBlaster Kits are shipped at room temperature.

2. Applications

GeneBlaster Kits help to achieve higher and longer levels of transgene expression. The GeneBlaster Kits are new formulations of chemical mixtures that significantly improve the gene expression level obtained by transfection and infection. Since the GeneBlaster reagents are a mixture of specific chemicals; they could affect cell phenotype to a certain degree. These reagents might increase cellular toxicity for certain cells and optimization may be required (see §3.3 Important remarks).

Since the application of the GeneBlaster Kits is **cell type dependent** three special formulations have been developed. Indeed, some cells will respond greatly to one GeneBlaster formulation whereas others are not responsive. In another way, some cells might respond to the trans-activation induced by all or several chemical mixtures (see tables below).

In the same way, the effects of the GeneBlaster Kits are also **dependent on the nature of the promoter** that control gene expression (see table 3). Indeed, according to the promoter (CMV, SV40 early gene promoter, ubiquitin, EF1a, HIV LTR, or tissues/genes specific promoters, etc.) the effect might differ in particular cell lines.

2.1 Cell Types & Promoters

The gene expression enhancement and persistence mediated by the GeneBlaster reagent is cell type dependent and promoter dependent. These formulations have been successfully tested on a variety of suspension and adherent cells (see table 1 & 2). If a particular cell type or cell line is not listed below, this does not mean that GeneBlaster Kits is not going to work.

Likewise, these formulations have successfully trans-activated a variety of gene expression under the control of particular promoters (see table 3). If a particular promoter is not listed below, this does not mean that GeneBlaster Kits is not going to work.

Table 1: Cell Types with increased gene expression levels with the GeneBlaster Reagents

GeneBlaster Reagents successfully increase the expression level of a β-galactosidase, GFP, Luciferase or others genes in the cell lines listed. Promoters successfully tested were CMV, SV40 early gene promoter, EF1a, HIV LTR, or glucocerebrosidase-induced promoter (see Table 3).

Cell Line	*Cell Type*	*Source*	*Ruby*	*Sapphire*	*Topaz*
293, HEK-293, 293-T	Transformed Embryonic Kidney	Human	√	√	√
A549, H538, H460	Non-small cell lung carcinoma	Human	√	√	√
A172, F-98	Glioblastoma	Human	√	√	√
ALL	Acute lymphoblastic leukemia	Human	N/A	N/A	√
Caco-2	Colon Adenocarcinoma	Human	√	√	N/D
CHP126	Neuroblastoma	Human	√	√	N/D
CHO	Epithelial-like (Ovary)	Hamster	√	√	N/A
HeLa, Hela-S3	Cervical Epithelial Carcinoma	Human	√	√	N/A
HT 29	Colon Adenocarcinoma	Human	√	√	N/D
HUVEC	Endothelial Cells (primary)	Human	N/A	√	N/A
Jurkat	T leukemia cells	Human	N/A	N/A	√
K-562	Myelogenous Leukemia	Human	N/A	N/A	√
MCF-7, Hs578T	Breast Adenocarcinoma	Human	√	√	N/A
NIH3T3	Fibroblasts	Mouse	√	√	N/A
P19	Teratocarcinoma	Mouse	√	√	√
PC-12	Pheochromocytoma (adrenal)	Rat	√	√	N/A
Primary	Peripheral Blood Lymphocytes Peripheral Blood Mononuclear cells	Human Mouse	N/A	N/A	√
Primary	Neurons	Rat	√	√	N/D
S2 TnB1-4	Drosophilia melanogaster Trichoplusia ni BTI	Insect	√	√	N/D

LEGEND: √: Works, N/A: Not Appropriate (due to toxicity and lowered gene expression) and N/D: Not Determined

Table 2: Gene expression controlled by CMV promoter in Cells Non-Responding to the GeneBlaster Reagents

For some of the cell lines listed, GeneBlaster may exhibit some cytotoxicity. In these cells, only a CMV promoter has been analyzed. Since GeneBlaster effect is promoter dependent, other promoter might be trans-activated by the chemical cocktails.

Cell Line	*Cell Type*	*Source*
B16F10	Melanoma	Mouse
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-7	Fibroblast (Kidney)	Green Monkey
CV-1	Fibroblast-like (Kidney)	Monkey
HepG2	Hepatoma	Human
MDCK	Normal -Kidney	Canine

Table 3: Promoters sensitive to trans – activation mediated by the GeneBlaster Reagents

The effects of the GeneBlaster Kits are also dependent on the nature of the promoter that controls gene expression. The nature and degree of trans activation depend also the cell types used (see tables above).

Promoters	***Ruby***	***Sapphire***	***Topaz***
CMV	√	√	√
SV-40 Early gene promoter	√	√	√
EF1a	√	√	√
HIV LTR, MoMSV LTR, RSV LTR...	√	√	√
MAT2A, IL2, MIP1alpha, IFNγ, NF-kappa B...	N/D	N/D	√
M human ChAT, H1(0), CTMP, MMTV, GPH alpha, Cytochrome P450, PLAP-1, Glucocerebrosidase-induced ...	√	√	N/D

LEGEND: √: Works, N/A: Not Appropriate (due to toxicity and lowered gene expression) and N/D: Not Determined

3. Protocols

3.1. General Considerations

- Select the suitable GeneBlaster reagent for favored cell types according to the Table 1.
- Briefly vortex the reagent before each use. If stored at -20° C, bring up to room temperature.
- Dilute the GeneBlaster reagent 100 times in the culture medium.

The instructions given below represent sample protocols that were applied successfully with a variety of cell lines and promoters. They can be used as guidelines to achieve very high gene expression level with minimal times. Optimal conditions do vary from cell line to cell line, promoter to promoter and the final dilution of the GeneBlaster Reagents might have to be adjusted to achieve best results. Therefore, we advise you to optimize few trans-activation parameters (concentration, incubation time, medium change...) if necessary.

3.2. General Protocol

1. Prepare the DNA/transfection reagent complexes (such as Magnetofection™ DreamFect™ or EcoTransfect reagents) according to the manufacturer's instruction or the viral titers as standard.

2. Add the complexes or viruses onto the cells growing in serum-free or serum-containing medium as standard culture conditions or as suggested by the manufacturer's protocol.

3. Incubate 4 hours.

4. Then, add the proper GeneBlaster to the cells (see tables 1, 3 & 4). Each GeneBlaster reagent supplied is 100X concentrated. The best method, to add the GeneBlaster to the cells in order to achieve the best mixing and to minimize cytotoxicity, is to first dilute the GeneBlaster in serum-containing medium. Therefore, prepare serum-containing medium with GeneBlaster at 2 to 10 X concentration (i.e., 1:50 to 1:10 dilution). For example, if the cells are transfected in

1ml of medium, prepare 1 ml of 2X (1:50 dilution) or 250 µl of 5X (1:20 dilution) or 110 µl of 10X (1:10 dilution) GeneBlaster solution in serum-containing medium. Finally, transfer the culture medium containing GeneBlaster to the cells (final concentration is 1X).

- ***OPTION***. As an alternative, the GeneBlaster can be directly added to the cells at a 1:100 dilution (1X final concentration, i.e. 10 µl in 1 ml) and mixed immediately by swirling or very gentle pipetting. The mixing process is very important to avoid localized toxic effects. If the transfection is realized in serum-free medium, first add the serum-containing medium to the cells and next, add the GeneBlaster to 1X final concentration.

5. The next day and if required, add fresh growth culture media without GeneBlaster. If some toxicity is observed and to avoid potential toxic effects, 24 hours post-transfection proceed to a medium change (discard the GeneBlaster -containing medium and replace by a fresh complete growth medium without GeneBlaster.

6. Cultivate the cells under standard conditions until evaluation of gene expression. The gene expression analysis can be monitored and assayed 24 to 72 hours following transfection or infection. This depends on the cell type, reporter gene and promoter activity.

Stable Transfection: the GeneBlaster reagents can also be used to produce stably transfected or transduced cells. 48 hours post-transfection or 24 hours post-infection replace old growth medium by fresh medium containing the appropriate antibiotics for selection.

Table 4: Recommended Uses for the GeneBlaster Reagents

Cells	Ruby	Sapphire	Topaz	Fold Improvement
293, HEK-293	++	++	++	**2-3X**
A549	++	++	N/D	**4-5X**
Caco-2	++	N/D	N/D	**2-4X**
HeLa	+++	++	N/A	**5-10X**
HT 29	+	N/D	N/D	**4-5X**
HUVEC	N/A	+	N/A	**2-3X**
Jurkat	N/A	N/A	+++	**5-10X**
K-562	N/A	N/A	+++	**5-15X**
MCF-7	++	++	N/A	**2-10X**
NIH3T3	++	+	N/A	**N/D**
P19	+++	+	++	**2-3X**
PC-12	++	+++	N/A	**3-10X**

LEGEND: + Works Well**, ++** Works Better, **+++** Works Best, N/A Not Appropriate (due to toxicity and lowered gene expression) and N/D: Not determined.

3.3. Important Remarks

- **For all GeneBlaster Kits**

Although the team of OZ Biosciences has carefully designed and optimized the GeneBlaster formulations for a number of cells, additional adjustment and optimization might be required for other cells to minimize toxicity and to enhance gene expression level after transfection or infection. We advise testing various dilutions of the GeneBlaster from 50X to 250X dilutions to attain the best results.

- **Caution!**

For some cell types such as B16-F0, BHK-21, CHO-K1, COS-7, CV-1, Hep-G2 & MDCK (especially if a CMV promoter controls the gene of interest) we do not recommend the use of the GeneBlaster Reagents. The chemicals either have no effect on the expression level of the gene controlled by a CMV promoter or on the transfection/infection efficiency or increase toxicity. Consequently, in function of the cell, the promoter and the concentration of GeneBlaster the chemicals mixture may induce inferior transfection efficiency and elevate toxic effects.

- **For GeneBlaster Ruby & Sapphire**
  - ✓ For handiness, GeneBlaster Ruby & Sapphire can be transfer to the cell culture medium at different times during the procedure. For example, they can be added to the cells concurrently with the DNA/transfection reagent complexes (or virus) or 4 hours post-transfection/post-infection (see section 3.2). Contrary to Topaz, Ruby & Sapphire formulations are not efficient if added 24 hours after transfection.

  - ✓ These two formulations can be relatively toxic for some cells. Consequently, the culture medium may need to be changed the next day with fresh GeneBlaster -free culture medium.

- **For GeneBlaster Topaz**

For convenience, GeneBlaster Topaz Reagent can be added to the cell culture medium at different times during the protocol. It can be added to the cells simultaneously with the DNA/transfection reagent complexes (or virus) or 4 hours post-transfection/post-infection (see section 3.2) or 24 hours after transfection/infection.

- **The GeneBlaster Emerald**

is the latest formulation of additives that significantly improve the number of transfected neurons and the gene expression level obtained with any viral and non-viral gene delivery systems such as Magnetofection™ (NeuroMag or CombiMag) or DreamFect™ Gold transfection reagents. It is suitable with all commercially available transfection reagents. The application of the GeneBlaster Emerald is specific to primary neurons. GeneBlaster Emerald is extremely easy to use: simply add the appropriate volume to your culture medium and boost neuronal transfection efficiency.

4. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
SilenceMag *(for siRNA application)*
NeuroMag *(dedicated for neurons)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
Viro-MICST (*to optimize viral transduction and purification*)
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for retrovirus and Lentivirus)*
AdenoMag *(for Adeno viruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
VeroFect *(for Vero cells)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)*
3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ 25µg pVectOZ-SEAP 25µg
ASSAY KITS
Bradford – Protein Assay Kit β-Galactosidase assay kits (CPRG/ONPG) X-Gal Staining Kit
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g X-Gal powder 1g

Purchaser Notification

Limited License

The purchase of this product 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). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the purposes 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.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us.

Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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 the 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

This product 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