# GeneBlaster™
# INSTRUCTION MANUAL


*Gene*

OZ BIOSCIENCES

# 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] | 3 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 |

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

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

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The art of delivery systems

# 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 Selection Kit**. This kit is a convenient assortment of the three 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 | 3 x 1.5 mL [1] | 1 red, 1 blue, 1 yellow |
| 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)

**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 with gel pack (+4°C) |

# 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. An updated list of cells successfully tested is available on our website: www.ozbiosciences.com.

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. An updated list of responsive promoters is available on our website: www.ozbiosciences.com

**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<br>Peripheral Blood Mononuclear cells | Human<br>Mouse | N/A | N/A | √ |
| Primary | Neurons | Rat | √ | √ | N/D |
| S2<br>TnB1-4 | Drosophilia melanogaster<br>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, IFN□, 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.

# 4. Related Products

| Description | Reference |
|---|---|
| **Magnetofection Technology** | |
| Super Magnetic Plate | MF10000 |
| Magnetic Plate 96-magnets | MF10096 |
| PolyMag 1mL *(for all nucleic acids)* | PN31000 |
| CombiMag 1mL *(to boost transfection reagent)* | CM21000 |
| SilenceMag 1mL *(for siRNA application)* | SM11000 |
| ViroMag 1mL *(for viral application)* | VM41000 |
| ViroMag R/L 1mL *(for lenti- & retro-viruses)* | RL41000 |
| NeuroMag 1mL *(for transfection of neurons)* | NM51000 |
| SelfMag Amino Kit | SA10000 |
| SelfMag Carboxy Kit | SC20000 |
| **Protein Delivery Systems** | |
| Ab-DeliverIN 1 mL | AI21000 |
| Pro-DeliverIN 1 mL | PI11000 |
| **Tee-Technology (lipid-based reagents)** | |
| Lullaby siRNA transfection reagent | LL71000 |
| DreamFect Gold Transfection reagent 1mL | DG81000 |
| EcoTransfect Transfection Reagent 1mL | ET11000 |
| VeroFect Transfection Reagent 1mL | VF61000 |
| FlyFectin Transfection Reagent 1mL | FF51000 |
| **Gene & Protein Tools** | |
| Bradford – Protein Assay Kit | BA00100 |
| β-Galactosidase (ONPG) assay kits | GO10001 |
| β-Galactosidase (CPRG) assay kits | GC10002 |
| X-Gal Staining Kit | GX10003 |

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