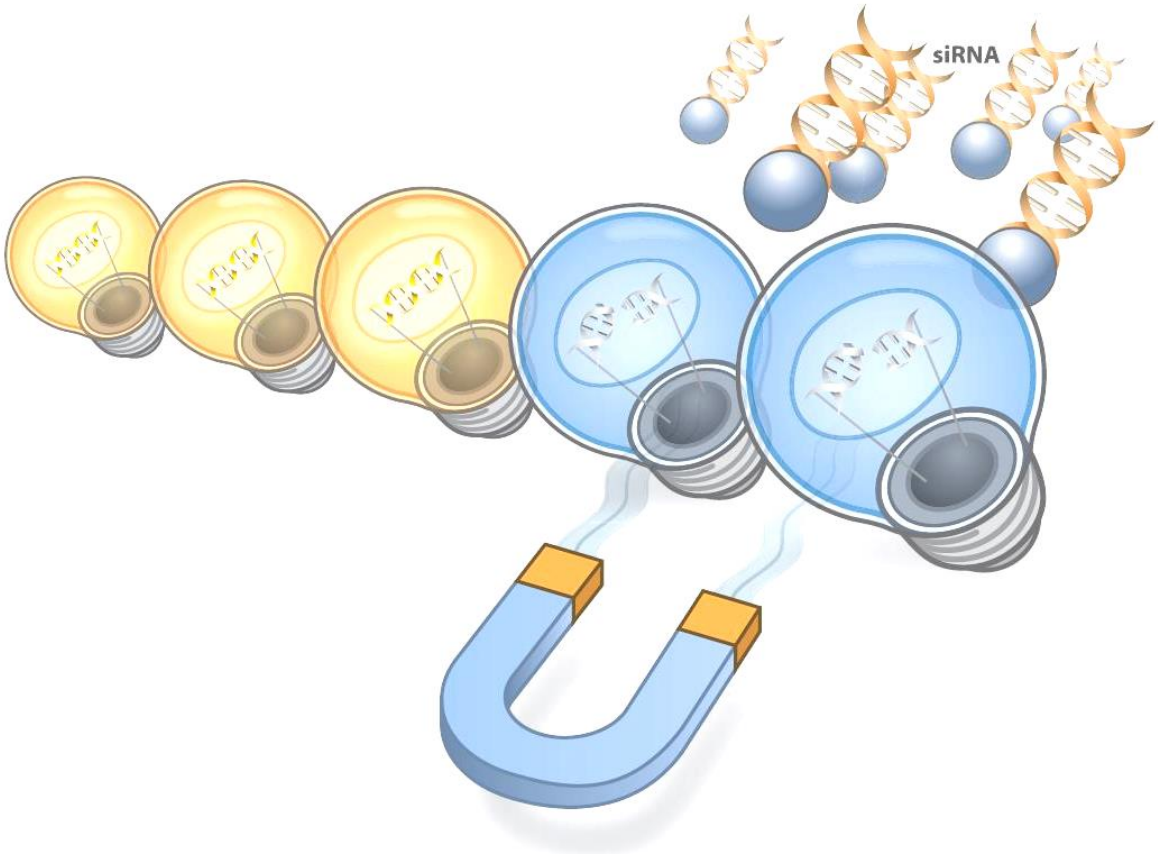
Magnetofection™ SilenceMag

INSTRUCTION MANUAL



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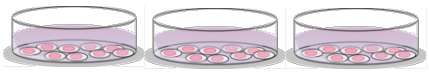
SilenceMag™ Short Protocol

To find your ideal silencing conditions with SilenceMag™, we suggest to test increasing doses of siRNA (or miRNA): from 10 to 50nM per well.

1 — **Seed cells to be at 70% confluent the day of transfection**



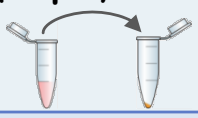
2 — **Prepare 3 tubes of siRNA (with different amounts of nucleic acids)**

96 well plate	24 well plate	6 well plate
10 nM/20nM/50nM in 50µL DMEM	10 nM/20nM/50nM In 100µL DMEM	10 nM/20nM/50nM In 200µL DMEM

3 — **Prepare 3 tubes of SilenceMag™ (with different amounts of reagent)**

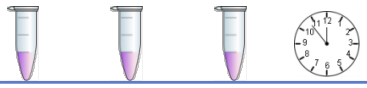
96 well plate	24 well plate	6 well plate
0.5µL/1µL/1µL in an empty microtube	1µL/2µL/3µL in an empty microtube	4µL/8µL/10µL in an empty microtube

4 — **Mix each tube of siRNA (step 2) to each tube of SilenceMag™ (step 3)**



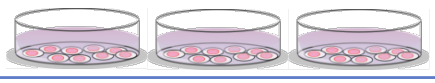
96 well plate			24 well plate			6 well plate		
siRNA		*SilenceMag™*	*siRNA*		*SilenceMag™*	*siRNA*		*SilenceMag™*
10nM	+	0.5µL	10nM	+	1µL	10nM	+	4µL
25nM	+	1µL	25nM	+	2µL	25nM	+	8µL
50nM	+	1µL	50nM	+	3µL	50nM	+	10µL

5 — **Incubate 20 min at room temperature**

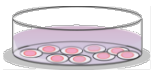


6 — **Distribute each mix onto the cells, and incubate the cells 15 min on the magnetic plate**

7 — **Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene silencing**



8 — **Choose the best ratio siRNA:SilenceMag™**



SilenceMag™

Instruction Manual

SilenceMag™ is a new reagent based on the Magnetofection™* technology specifically designed for all siRNA applications

List of SilenceMag™ Kits

Catalog Number	Description	Volume (µL)	Number of transfections[8] / 24 well plates	Number of transfections[8] / 96 well plates
SM10200	SilenceMag	200	200	≥ 400
SM10500	SilenceMag	500	500	≥ 1000
SM11000	SilenceMag	1000	1000	≥ 2000
SM13000	SilenceMag	3x1000	3000	≥ 6000
KM30300	Selection Kit siP [1]	200 +2x100	200 + 2x 100	400 + 100
KM30350	Selection Kit siC [2]	200 + 100	200 + 100	400 + 100
KM30400	Super Selection Kit [3]	200 + 3 x 100	200 + 3 x 100	400 + 2 x 100
KC30396	siRNA Starting Kit [4]	200	200	400
KC30496	Super Starting Kit [5]	200 + 3 x 100	200 + 3 x 100	400 + 2 x 100
KC30300	siRNA Starting Kit [6]	200	200	400
KC30400	Super Starting Kit [7]	200 + 3 x 100	200 + 3 x 100	400 + 2 x 100
MF10000	Super Magnetic Plate	N/A	N/A	N/A
MF10096	96-Magnets, Magnetic Plate	N/A	N/A	N/A

[1] Contains 1 vial of SilenceMag/1 vial of PolyMag/1 vial of PolyMag Neo - [2] Contains 1 vial of SilenceMag/1 vial of CombiMag - [3] Contains 1 vial of SilenceMag/1 vial of PolyMag /1vial of PolyMag Neo/1vial of CombiMag - [4] Contains 1 vial of SilenceMag and a 96-magnets Magnetic Plate - [5] Contains 1 vial of SilenceMag/1 vial of PolyMag/1 vial of PolyMag Neo/ 1 vial of CombiMag and a 96-magnets Magnetic Plate -

[6] Contains 1 vial of SilenceMag and a Super Magnetic Plate - [7] Contains 1 vial of SilenceMag/1 vial of PolyMag/1 vial of PolyMag Neo/1 vial of CombiMag and a Super Magnetic Plate - [8] Number of transfection given for a concentration of 10nM siRNA.

* Patent Pending

1. Technology

1.1. Description

Congratulations on your purchase of the **SilenceMag™** reagent!
Issue from our Magnetofection™ technology, **SilenceMag** was specifically designed to deliver siRNA inside cells.

Magnetofection™ is a novel, simple and highly efficient method to deliver nucleic acids into cells. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the nucleic acids towards, possibly even into, the target cells. In this manner, the complete applied nucleic acid dose gets concentrated on the cells within a few minutes. **SilenceMag** is a unique and specific reagent dedicated to the intracellular delivery of siRNA, dsRNA or shRNA. This reagent demonstrates an exceptionally high efficiency to target such ribonucleotides into cells.

SilenceMag is the most efficient and powerful siRNA delivery systems due to unique features:

1. High percentage of cells transfected compared to standard transfections.
2. Concentration of all nucleic acid dose on the cells very rapidly
3. Very high amount of siRNA delivered in cells
4. Higher gene silencing efficacy even at very low dose of siRNA (from 1 to 10nM). Depending on the target, gene knockdown can be seen even below 1nM.

Based upon a validated and recognized magnetic drug targeting technology this innovative method is:

- Highly Efficient
- Suitable for all siRNA applications (co-transfection, endogenous gene silencing)
- Economical (save materials). Use 10 to 100 times less siRNA and achieve high gene silencing
- Simple & rapid
- Universal (primary cells, hard-to-transfect cells and cell lines)
- Serum compatible & Non toxic

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.2 mL of **SilenceMag** good for 200 assays in a 24-well plate with 10nM of siRNA.
- 1 tube containing 0.5 mL of **SilenceMag** good for 500 assays in a 24-well plate with 10nM of siRNA.
- 1 tube containing 1 mL of **SilenceMag** good for 1000 assays in a 24-well plate with 10nM of siRNA.
- 3 tubes containing 1 mL each of **SilenceMag** good for 3000 assays in a 24-well plate with 10nM of siRNA.

Stability and Storage

Storage +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. Magnetofection kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE MAGNETIC NANOPARTICLES!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**

Shipping condition Room Temperature

2. Applications

2.1. siRNA Mediated Gene Silencing

RNA interference is a powerful technique to shut down genes expression in cells and organisms. This silencing effect constitutes a very helpful tool to study gene's function and a promising approach for new therapeutic treatments. Short RNA duplexes (siRNA: small interfering RNA, shRNA: small hairpin RNA and dsRNA: double strand RNA) are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein production. **SilenceMag** introduces the siRNA duplexes in a variety of cells with a very high efficiency leading to exceptional knockdown effects with low doses of siRNA.

2.2. Cell Types and Targets

Magnetofection™, the technology used with **SilenceMag**, is generally applicable on numerous cell types. This technology has been tested successfully on a variety of immortalized and primary cells and multiple targets. If a particular cell or target is not listed, this does not imply that **SilenceMag** is not going to work.

Cell Line	*Cell Type*	*Source*
293, A293 HEK-293 293-T, 293-EBNA	Transformed Embryonic Kidney	Human
A549	Non-small cell lung carcinoma	Human
B16F10	Melanoma	Mouse
BHK-21	Kidney	Hamster
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblast (Kidney)	Green Monkey
CT-26	Colon Carcinoma	Mouse
CV-1	Fibroblast-like (Kidney)	Monkey
HaCaT	Immortalized Keratinocytes	Human
HeLa	Cervical Epithelial Carcinoma	Human
Hep2	Laryngeal Epithelium	Human
HepG2	Hepatoma	Human
HT1080	Fibrosarcoma	Human
HUVEC	Endothelial Cells (primary)	Human
MCF-7	Breast Adenocarcinoma	Human
MDCK	Normal -Kidney	Canine
N2A	Neuroblastoma	Mouse
NIH3T3	Fibroblasts	Mouse
PC-12	Pheochromocytoma (adrenal)	Rat
Primary Airway Epithelium		Porcine, Human
Primary HUVEC Endothelial Cells		Human
Primary Keratinocytes		Human, Mouse

Targeted reporter genes (co-transfection and stably transfected cells): GFP, Luciferase, Lac Z...
Targeted endogenous genes: GAPDH, Lamin, Transcription factors...

3. SilenceMag / Magnetofection™ Apparatus

As for all Magnetofection™ reagents, **SilenceMag** requires an appropriate magnetic field. A magnetic plate especially designed for Magnetofection is provided to exert this specific magnetic field. Its special geometry produces a strong magnetic field that is suitable for all cell culture dishes (T-75 flasks, 60 & 100 mm dishes, 6-, 12- 24-, 48- and 96-well plates).

4. Protocol

4.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cells. Our R&D team has extensively tested and optimized **SilenceMag** reagent in order to provide you with the simplest, straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines to obtain good data quickly and if necessary, we advise you to optimize the transfection parameters in order to achieve the best effects. Optimal conditions do vary from cell to cell and are highly dependent upon both the siRNA sequence (shRNA or dsRNA) and the gene targeted. Consequently, the amount, concentration and ratio of the individual components (siRNA and reagents), the time course and the number of cells may have to be adjusted to get the best results. Several optimizations protocols are available in the Appendix. The siRNA optimal concentration required to achieve the best gene silencing effect depends highly on the cells, target and siRNA sequence; consequently, we suggest to first test a range of siRNA concentration from 1 to 40nM.

4.2. General Protocol

It is recommended to seed or plate the cells the day prior transfection, however cells can also be prepared few hours before the transfection. Suspension cells should be prepared in the adequate vessel just before the transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Best results are achieved if cells are at least 50-70 % confluent at the time of Magnetofection (see the suggested cell number in the table below).

Table 1: Recommended number of cells to seed.

Culture vessel	Number of adherent cells	Number of suspension cells	Cell overlay volume
96-well	$6 - 12 \times 10^3$	$4 - 8 \times 10^4$	100 µL
24-well	$4 - 8 \times 10^4$	$25 - 50 \times 10^4$	400 µL
12-well	$8 - 15 \times 10^4$	$5 - 10 \times 10^5$	900 µL
6-well	$2 - 4 \times 10^5$	$1 - 2 \times 10^6$	1800 µL
60 mm dish	$5 - 10 \times 10^5$	$2 - 5 \times 10^6$	3800 µL
90 – 100 mm dish	$10 - 20 \times 10^5$	$4 - 10 \times 10^6$	7800 µL

According to the standard protocol, the siRNA (shRNA or dsRNA) / **SilenceMag** mixtures are prepared in medium without serum and supplement or in physiological saline because serum may interfere with vector assembly. These mixtures are then added to the cells that are covered with complete medium. Therefore, the addition of this cocktail will result in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium. Although a medium change after Magnetofection is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration.

4.3. SilenceMag™ Procedure

The protocol is very straightforward. Please refer to the tables below for specific amount of the respective compounds and transfection volume. For instance:

- Use 1 L of **SilenceMag** for 10nM siRNA final concentration in a 24-well plate (volume 500 l)
- Use 4 L of **SilenceMag** for 10nM siRNA final concentration in a 6-well plate (volume 2 mL)

1) Plate the cells the day before transfection or just before transfection in your appropriate tissue culture dish and volume of culture medium as suggested in Table 1.

2) Dilute the siRNA to 100 µL (or 200µL) with culture medium **without** serum and supplement (such as DMEM) (see Table 2 for siRNA dilution procedure).

Table 2: Suggested dilution procedure and amount of siRNA to test:

Culture vessel	96-well		24-well		12-well		6-well	
Dilution serum-free medium	100 µL		100 µL		100 µL		200 µL	
Amount of siRNA (1 µM stock)*								
Final siRNA concentration	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**
1 nM	0.2	2.7	0.5	6.75	1	13.5	2	27
2 nM	0.4	5.4	1	13.5	2	27	4	54
5 nM	1	13.5	2.5	33.75	5	67.5	10	135
10 nM	2	27	5	67.5	10	135	20	270
20 nM	4	54	10	135	20	270	40	540
50 nM	10	135	25	337.5	50	675	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

3) Before each use, vortex the **SilenceMag** vial. Add directly the **SilenceMag** reagent to a microtube according to the volumes shown in Table 3 (below). If required, **SilenceMag** can only be diluted with deionized water. Do not dilute the reagent in serum and supplement-free medium.

Table3: Recommended amount of **SilenceMag** per nM of siRNA used:

Culture vessel	96-well	24-well	12-well	6-well
Final transfection volume	200 µL	500 µL	1 mL	2 mL
Amount of SilenceMag				
Final siRNA concentration				
1 nM	0.5 µL	1 µL	2 µL	4 µL
2 nM	0.5 µL	1 µL	2 µL	4 µL
5 nM	0.5 µL	1 µL	2 µL	4 µL
10 nM	0.5 µL	1 µL	2 µL	4 µL
20 nM	1 µL	2 µL	4 µL	8 µL
≥ 50 nM	1 µL	3 µL	6 µL	10 µL

4) Add the 100 or 200 µL of siRNA-diluted solution to the **SilenceMag** tube and mix immediately 4–5 times by vigorous pipetting.

5) Incubate the mixture (siRNA/SilenceMag) 20 minutes at room temperature.

6) Add the 100 µL (or 200 µL) of complexes drop by drop directly onto the cells. The total transfection volumes per well (culture medium + **SilenceMag** mixture) are shown in the Table 3.

Note: For some cells, serum-free condition for the first 3 hours of incubation might lead to better gene silencing. However, in most assays, siRNA delivery has been realized in culture medium with serum.

7) Place the cell culture plate upon the magnetic plate for 15 minutes.

8) Remove the magnetic plate.

9) Cultivate the cells under standard conditions until evaluation of the gene silencing. Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection. We recommend 24h and 72h for RNA and protein knockdown analyses, respectively.
Note: Optionally a medium change can be performed 8-24h after the transfection if your cells are sensitive to serum/supplement concentration.

5. Appendix

5.1. Critical Parameter for best performance

1) Cell culture conditions: Best results are achieved when cells are 50–70 % confluent at the time of the transfection. If necessary, you can wash the culture medium containing the transfection mixture after 8-24 hours and replace it by fresh medium.
2) siRNA concentration. We often observed good siRNA effects at very low concentrations from 0.1 to 5 nM. However the efficiency may depend on the cell line, the target (half life, expression level...) and the siRNA used. Consequently, we suggest you to start by testing a range of siRNA concentrations in order to obtain the best experimental conditions.
3) Saving materials. In order to use less siRNA during your experiments you can also reduce the total transfection volume for the first 24 hours and then add more, fresh complete medium to maintain the cells in good conditions.
4) Time course. The gene silencing time course depends on the amount/concentration of siRNA used. Indeed, with high quantity of siRNA, very efficient gene expression knockdown can be observed at earliest time point such as 16 or 24 hours. In contrast, with low siRNA concentration gene silencing require longer incubation such as 48 or 72 hours.

5.2. Protocol Optimization

In order to get the best out of **SilenceMag™**, several parameters can be optimized:

- siRNA dose used, which strongly depends on the efficiency and specificity of your siRNA
- Ratio of **SilenceMag** to siRNA
- Cell type and cell density
- Incubation time

OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedures described above (section 4).

1) Start by optimizing the siRNA dose with the fixed ratio of **SilenceMag** /siRNA that has been previously optimized.

2) Thereafter, change the ratio **SilenceMag** / siRNA. To this end, use a fixed amount of siRNA and vary the amount of **SilenceMag** from 2 times less up to three times more than the suggested amount detailed in the Table 3. **For instance,** from 0.5 to 3µL of **SilenceMag** in a 24-well plate or from 2 to

12µL of **SilenceMag** in a 6-well plate for 10 nM siRNA. The ratio of **SilenceMag** / siRNA can be changed by doubling or multiplying the volumes of the reagents used. Similarly, the reagents can be pre-diluted in deionized water and aliquots of the resulting dilutions are incubated with siRNA.

3) After having identified the correct quantity of **SilenceMag** and siRNA, you could pursue the process by optimizing the cell number (density) and time course of your experiment.

5.3. Suspension Cells Protocol

1. The composition and dilution series are performed exactly as described in the section 4.3 from steps 1 to 4.

2. While **SilenceMag** and siRNA incubate (step 5 in the section 4.3) dilute the cells as suggested Table 1 in serum containing medium (with or without serum- or supplement; depending on the cell type and sensitivity of cells towards serum-free conditions) and perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles:

    a. Seed the cells on polylysine-coated plates and use the protocol for adherent cells. **OR**
    b. Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells. **OR**
    c. Mix cell suspension with 20-30 µL of *CombiMag* (Magnetofection) reagent per 1 ml of cell suspension and incubate for 10 - 15 minutes. Then, distribute the cells to your tissue culture dish placed upon the magnetic plate and incubate for 15 more minutes.

3. Add the **SilenceMag** / siRNA mixture to the cells (while keeping the cell culture plate on the magnetic plate if you proceeded using the method c described just above).

4. Incubate for 15 minutes.

5. Remove culture plate from magnetic plate.

6. Cultivate cells as desired until the evaluation of gene silencing.

5.3. Quality Controls

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

Components	Standard Quality Controls
SilenceMag	1. Quality and size homogeneity of the magnetic nanoparticles. 2. Stability of the magnetic nanoparticles formulations. 3. Knockdown efficacies of EGFP in HeLa cells stably expressing EGFP. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.
Magnetic Plate	1. Tests of solidity and Test of the magnetic field force

6. 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*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds 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 / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
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.

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