CELL guidance systems

User Guide

ExoFLARE™

Exosome Tracking System

Cat EX30X/EX40X

Protocol Version 1.0

Contents

Storage

Upon receipt, store dye at room temperature, avoid contact with light.

Store the other components at 4°C.

Correctly stored components are stable until the date shown on the packaging.

Product Components

ExoFLARE™ kit

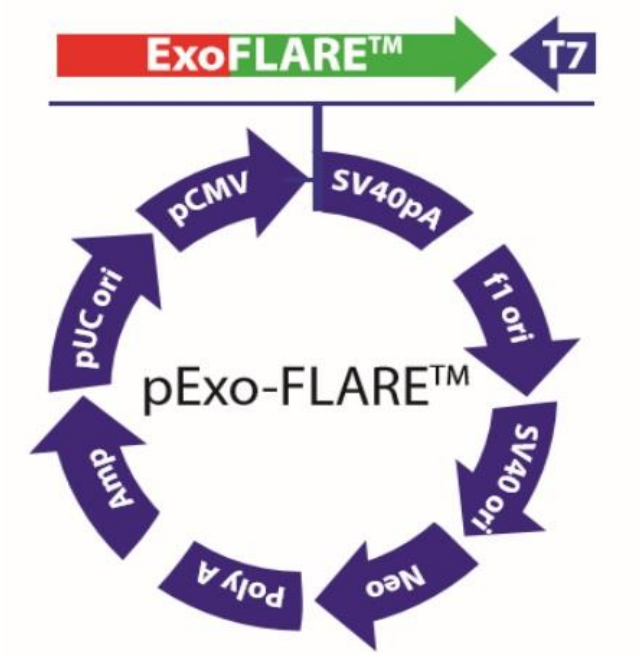
- 20 µg ExoFLARE™ plasmid, 1 vial
- 96 rxn (96-well format) Cell permeable/impermeable ExoFLARE™ dye, 1 vial
- 100 µl/200 µl Solvent A, 1 vial
- 2 ml PBS, 1 vial

Product Information

The ExoFLARE™ system utilizes a combination of FLARE (FLuorescence Activating Response Element) protein tag together with a pro-fluorophore dye. Neither the protein nor dye fluoresce in isolation. When the protein binds to the dye, it causes a change in structure of the dye which results in fluorescence. The dye and protein form an unstable bond with continuous turnover of the dye. This results in a sustained fluorescence without the levels of photo-bleaching associated with regular fluorescent proteins and dyes. This allows ExoFLARE™ tagged vesicles to be monitored for extensive periods to allow tracking of exosome movement.

General Information

ExoFLARE™ plasmid map



Vector components

Component	Description
CMV promoter	Enables efficient, high-level expression of the ExoFLARE™ tagged protein.
Tetracycline operator (2X TetO2)	Two tandem 19 nucleotide repeats which serve as binding sites for Tet repressor homodimer
ExoFLARE™ tagged protein	The protein tag will interact with the ExoFLARE™ dye and generate an intense fluorescent signal
T7 promoter (complementary strand)	Provides competent *in vitro* transcription in the antisense orientation
SV40 polyadenylation region	Provides effective transcription termination and polyadenylation of mRNA
f1 origin	Permits retrieval of single-stranded DNA
SV40 early promoter and origin	Enables high-level expression of the neomycin resistance gene along with episomal replication in cells expressing the SV40 large T antigen
Neomycin resistance gene	Selects for stable transfectants in mammalian cells
Polyadenylation signal	Provides efficient transcription ending and polyadenylation of mRNA
Ampicillin resistance gene (β-lactamase)	Selects for transformants in *E. coli*
pUC origin	Allows high-copy number replication and proliferation in *E. coli*

ExoFLARE™ dye re-suspension

1. Add 20 µl Solvent A to the amber vial containing the ExoFLARE™ dye.

2. Vortex vigorously for 30-60 seconds to ensure complete re-hydration of the compound.

3. Add 1980 µl of PBS to the amber vial containing the ExoFLARE™ dye.

4. Vortex for 5 seconds.

The resulting ExoFLARE™ dye solution is a 10x stock.

Protocol

A. Transfection

1. Ideally, seed cells on a glass surface which offers an even focal plane, this enables effective subsequent imaging.

2. Once the cells reach approximately 70% confluency, proceed with the transfection method of choice following the transfection reagent or transfection instrument manufacturer's instructions.

B. Imaging

1. Prepare 96 well plates containing 180 µl of cell culture medium in each well.

2. Add 20 µl of 10x ExoFLARE™ dye solution to each well in order to obtain a working concentration of 100 nM. Incubate for 2-5 minutes.

3. Start imaging. The ExoFLARE™ dyes have an excitation peak at 640 nm and emission maximum at 680 nm (a Cy5-like spectrum).

Useful Notes

Solvent A consists of a 25%-75% DMSO-PBS solution.

After addition of the dye to the cell culture, the working concentration of DMSO will be 0.025%, this is well below acceptable levels and will not induce cytotoxicity.

For cell culture surface, we recommend CELLview™ slides which can be purchased from Greiner Bio One.

Purchaser Notification

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