Product Analysis Certificate

PRODUCT **EV-Entry System**

CATALOG # **EVEN105A-1, EVEN110A-1**

STORAGE **EV-Entry regents A and B store at -20°C**

SHELF LIFE **12 months from date of receipt with proper storage**

SHIPPING **Dry Ice (-80°C)**

DESCRIPTION

Extracellular vesicles (EVs), including exosomes, are nanocarriers used by cells to transport RNA and protein signals and are central to intercellular communication. EVs can be added to recipient cells to deliver exogenous cargo. The EV-Entry system not only enhances the rate of uptake of EVs by the recipient cells but also increases the offloading of the transported cargo into the cytoplasm of the recipient cells. EV-Entry boosts the delivery of both RNA and protein cargo.

The EV-Entry system is comprised of Reagent A and B components that are mixed together with EVs or exosomes just prior to use. The protocol takes less than an hour and is highly efficient at enabling EVs and exosomes to deliver cargo to recipient cells.

PACKAGE CONTENTS

Description	catalog#	Size
EV-Entry Reagent System Kit	EVEN105A-1	5 Reactions
EV-Entry Reagent System Kit	EVEN110A-1	10 Reactions

KIT COMPONENTS

Component	Amount 5 rxn	Amount 10 rxn
Reagent A (20X)	25 ul	50 ul
Reagent B (1X)	10 ul	20 ul

PROTOCOL

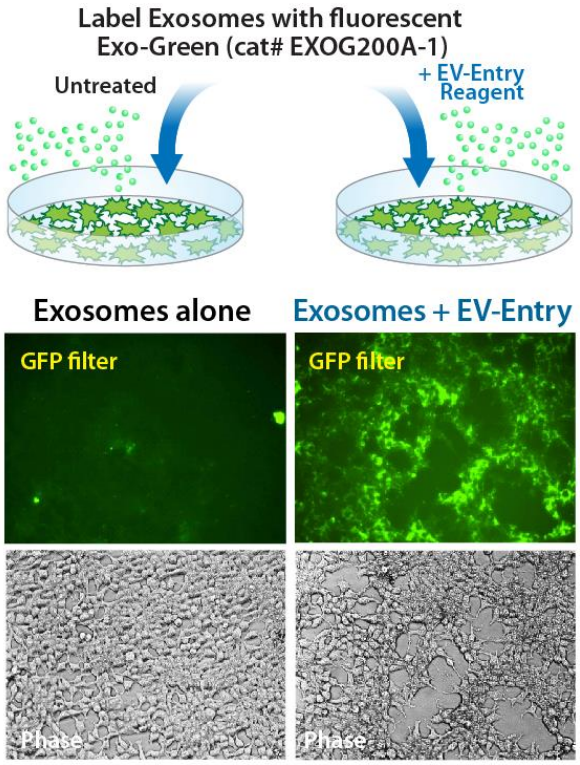
NOTE: We recommend using about 10^7 EVs/exosomes (50-100 ug protein) to add to approximately 200,000 recipient cells. This can be scaled up or down accordingly.

1. Resuspend EV/exosome pellet in 93 uL sterile DMEM media (without FBS and antibiotics)
2. Add 5 uL 20X EV-Entry Reagent A and 2 uL 1X EV-Entry Reagent B to the EV/exosome suspension and mix well by pipetting up and down 3 times.
3. Incubate the exosomes with EV-Entry reagents for 45 minutes at room temperature.
4. Add the entire EVs/exosomes suspension to target cells for enhanced cargo delivery.

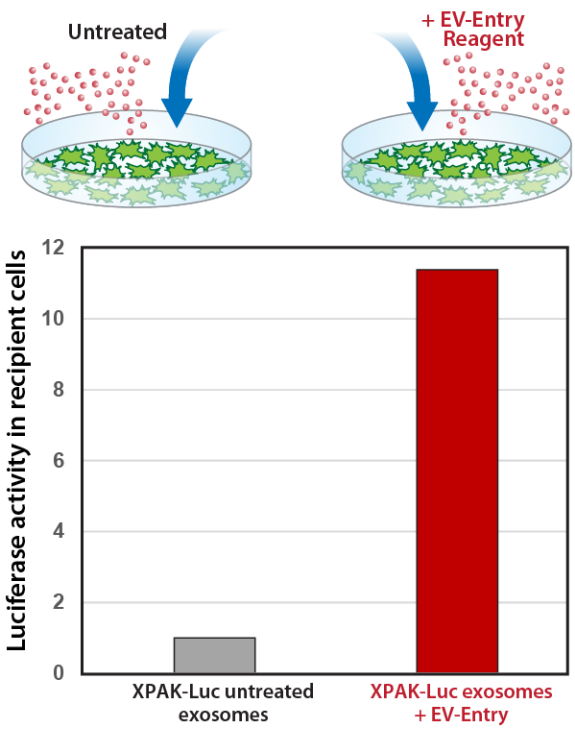
Enhancement in exosome entry can be visualized as early as 3h after incubation of exosomes on recipient cells. Some cell types however may take longer for difference in internalization and delivery to become evident.

SAMPLE DATA

Exosomes were labeled with ExoGreen (EXOG200A-1), a dye that binds to exosomal proteins and added on HEK cells with or without the EV-Entry reagent. Cells were imaged 18h post incubation of labeled exosomes with the cells. Cells that received exosomes with the EV-Entry reagent showed remarkably enhanced uptake of exosomes as well as release of exosomal cargo in the cytoplasm as demonstrated by the staining pattern of exosomal proteins in the recipient cells.



HEK 293 cells were transfected with XPack-Luciferase (XPAK732PA-1). Exosomes from transfected cells were harvested and added on to naïve HEK 293 cells with or without the EV-Entry reagent. After 18h incubation, target cells were washed multiple times with PBS and lysed. Luciferase activity in the recipient cells was measured by luciferase assay. Cells that received XPack-Luciferase exosomes with the EV-Entry reagent demonstrated greater than 11-fold greater luciferase activity compared to untreated exosomes suggesting enhanced delivery of the enzyme to recipient cells with EV-Entry.



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