B18R-E3L RNA (human codon optimized for B18R and E3L)

Synthetic RNA

Cat. # SCR722

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack size: 10 µg

Store at -80 ºC

Data Sheet

Background

Simplicon™ is a novel system to effect immediate high sustained protein expression of multiple genes into transfected cells without the risk of genome integration. The technology employs a single, synthetic, polycistronic, self-replicating RNA based on the Venezuelan equine encephalitis (VEE) genome[1,2,3]. The Simplicon™ RNA contains only genes encoding the VEE RNA replication machinery while the structural proteins that are required to make an infectious particle have been removed and replaced with the transgenes of interest.

Introduction and replication of the Simplicon™ RNA is expected to elicit a strong interferon response in transfected cells. To suppress the IFN responses, a Vaccinia virus protein[4], B18R, is used for the original Simplicon™ technology. Recently, we found that another Vaccinia virus protein[4], E3L, also suppresses the IFN responses in Simplicon™ RNA expression. B18R neutralizes type I interferons by direct binding, while E3L inhibits the cytoplasmic signaling pathways of IFN responses. Therefore, B18R and E3L are both employed in the Simplicon™ Expression System and work collaboratively to suppress IFN responses. As a result, there is increased cell viability during RNA transfection and increased expression of the transgenes. The Simplicon™ Expression System works in human cells and is not expected to work in mouse cells. This is because the B18R does not effectively neutralize mouse interferon (IFN)-β.

The B18R-E3L RNA (human codon optimized for B18R and E3L) is a synthetic polycistronic mRNA that is co-transfected with the Simplicon™ RNA to suppress the IFN responses. B18R-E3L RNA can also be used to prepare B18R conditioned medium by transfection into HFFs (Cat. No. SCC058). B18R-E3L RNA is also available for co-transfection of any mRNAs to suppress the IFN responses and enable repeated transfection of mRNAs. Please refer to the User Guide for Simplicon™ Expression System located on our website (www.emdmillipore.com).

Storage & Handling of Kit Components

1. B18R-E3L RNA (human codon optimized for B18R and E3L): (CS224503) One (1) vial containing 10 µL of RNA (1 µg/µL). Store at -80°C.

For best recovery, quick-spin the vials after thawing on ice. Do not vortex. Aliquot into sterile, nuclease-free eppendorf tubes on ice and store at -80°C. Limit repeated freeze-thaw cycles.

Quality Control

- Discrete RNA band on RNA gel of appropriate size.
- 40-60% GFP-positive human foreskin fibroblasts (HFFs) with FACS analysis.

Representative Data

Figure 1. 5' capped RNA was in vitro transcribed. The poly (A) tail was added by an enzymatic poly (A) adenylation reaction. cDNAs for B18R and E3L were human codon optimized.

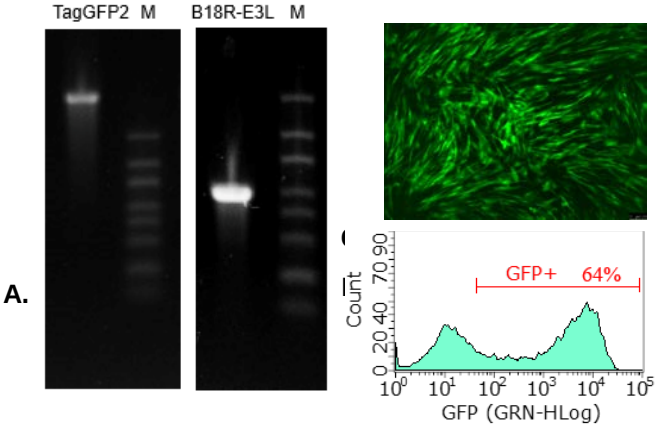

Figure 2. RNA gels and TagGFP2 expression in HFFs.
(A) RNA gels (Left): TagGFP2 Simplicon™ RNA (E3L), (Right): B18R-E3L RNA. Marker: 6, 4, 3, 2, 1.5, 1, 0.5, 0.2 kb. **(B, C)** TagGFP2 Simplicon™ RNA (E3L) and B18R-E3L RNA were co-transfected into HFFs with MessengerMax™ transfection Reagent. One day after transfection, GFP expression was analyzed by fluorescence microscopy and FACS.

References

1. Yoshioka, N., et. al. Cell Stem Cell 2013, 13(2):246-254.
2. Yoshioka N., and Dowdy SF. PLoS One. 2017;12(7):e0182018. doi: 10.1371/journal.pone.0182018. eCollection 2017.
3. Pegg G., *et al.* Drug Metabolism and Pharmacokinetics, Volume 33, Issue 1, Supplement, 2018; Pages S33-S34
4. Perdiguero B., and Esteban M. J Interferon Cytokine Res. 2009;29(9):581-98. doi: 10.1089/jir.2009.0073.

Rev.1.0/2018-04-20/SCR722VC

RELATED PRODUCTS

Cat #	Description
SCR720	TagGFP2 Simplicon™ RNA (E3L) Kit
SCR721	TagRFP Simplicon™ RNA (E3L) Kit
SCR723	B18R RNA (human codon optimized)
SCR724	Simplicon™ Cloning Vector (E3L)
SCR725	TagGFP2 Simplicon™ Plasmid (E3L)
SCR726	TagRFP Simplicon™ Plasmid (E3L)
SCR727	B18R-E3L Plasmid (human codon optimized for B18R and E3L)
SCR728	B18R Plasmid (human codon optimized)
GF156	B18R protein (produced from insect cells)
GF197	B18R protein (priduced from HEK 293 cells)

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