ProMab Biotechnologies, Inc.

Protocol

VIRAL PRODUCTION

OVERVIEW

TiterMax™ Reagent (cat. # VB100, ProMab Biotechnologies) is a novel cocktail of small molecules that can enhance viral production and is a powerful, broadly applicable reagent for effective virus packaging. It stably regulates the viral RNA packaging on the transcriptional level, which can greatly enhance production of either retro or lentiviral particles up to 10-fold. The easy-to-use protocol makes TiterMax™ Reagent well-suited for various scales of virus packaging.

PROCEDURE

Day 1: The day before transfection

1. Coat plates/dishes with 1x Gelatin for 30 min. Aspirate gelatin, and plate ~3-4 X 10^6 HEK 293T cells per 100-mm plate. Use 10 ml medium for each plate.
   Note: It is very important to have good single cells suspensions and to evenly distribute the cells.

Day 2: Transfection

Note: Prepare your transfection following manufacturer's protocol.Note: Prepare your transfection following manufacturer's protocol.

2. Prepare two tubes and add 0.5 ml DMEM to each tube. To one tube, add DNA mix (containing viral vector and packaging mix) and mix well by tabbing the tube. To the other tube, add NanoFect (Cat. no. NF100). Mix by tapping the tube.

Note: Incubate at room temperature (20–25 °C)for no longer than 5 min.

3. Transfer NanoFect-DMEM mixture into the DNA tube, pipette up and down for 2–3 times. Mix well by vortexing for 5-10 seconds.
4. Incubate for ~15 minutes at room temperature to allow for NanoFect/DNA complexes to self-assembly.
5. Add the NanoFect/DNA mix drop-- ‑ wise to the plate, gently rock the plate and place the plate back to the incubator.

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Day 3: Change medium and add TiterMax™

6. Replace supernatant with 10 ml fresh media and supplement with 20 l of TiterMax (500X). Return the plates to the cell culture incubator.

Day 4: Collect Virus

! **CAUTION:** Handle virus material with caution and avoid spills. Use bleach (10% final concentration) to decontaminate hazardous liquids for 30 min!

7. Collect the supernatant in a 50 ml conical tube and put in on ice. Centrifuge the supernatant at 1,000g for 10 min to remove cell debris (Preset the centrifuge to 4°C).
8. Filter the supernatant through 0.45 m filter. Transfer filtered supernatant to a sterile vessel and add 1 volume of cold Retrovirus/Lentivirus Precipitation Solution (4°C, Cat. # VC100 & VC200) to every 4 volumes of virus-containing supernatant.
Example: 5 ml Retrovirus/Lentivirus Precipitation Solution with 20 ml viral supernatant.
9. Mix well and refrigerate overnight.

Day 5: Concentrate virus

10. Centrifuge mixture at 1500g for 30 minutes at 4°C. After centrifugation, viral particles may appear as a beige or white pellet at the bottom of the vessel.
11. Discard supernatant. Spin down residual solution by centrifugation at 1500g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated viral particles in pellet.
12. Resuspend viral pellets in 1/10 to 1/100 of original volume using cold, sterile PBS or DMEM at 4°C. Aliquot in cryogenic vials and store at – 80°C until ready for use.

Note: TiterMax™ reagent can be removed by using viral concentration/purification procedures. The side effect of crude viral particles with TiterMax reagent on the expression of gene of interest has not been detected when used directly to transduce HEK 293T cells, but it may be various from cell line to cell line. It is advised to test the effect of TiterMax™ on the target cells beforehand.

DATA ON NEXT PAGE

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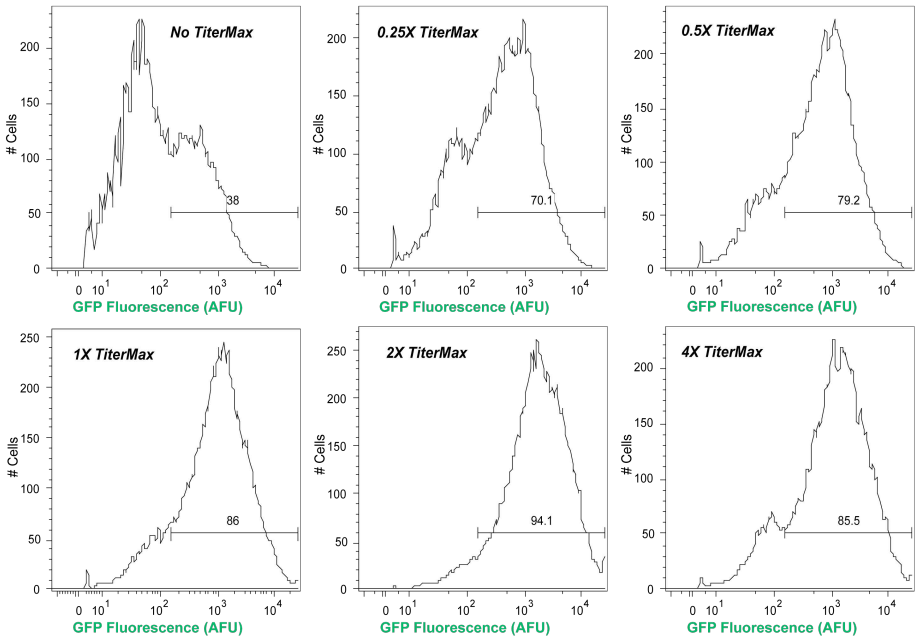

Figure 1. TiterMax™ Reagent enhances virus production up to 10-fold. Flow cytometry data were from the HEK 293T cells transduced by GFP retroviruses which were packaged in the absence and presence of TiterMax™.

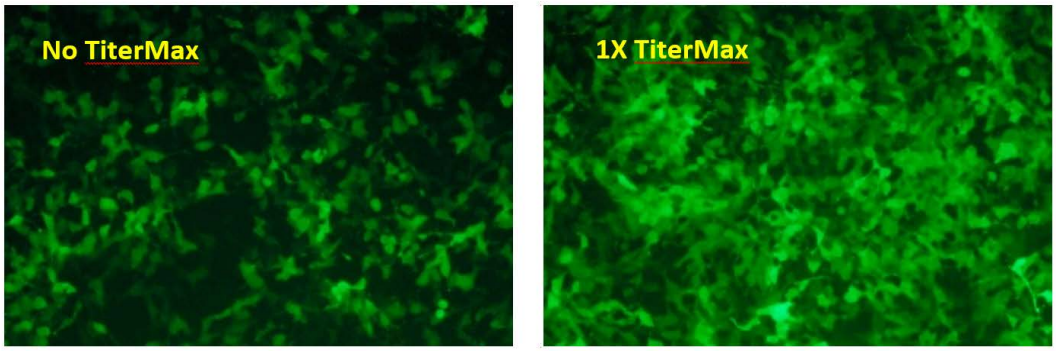

Figure 2. Both the number of GFP positive cells and GFP intensity measured by fluorescence microscopy were greatly enhanced when HEK 293T cells transduced by GFP lentiviruses which were packaged in the presence of TiterMax™.