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Data Sheet

YFP (Topaz) Lentivirus

Catalog #: 79989

Product Description
Topaz[1], a yellow fluorescence protein (YFP), is one of the brightest and thermally stable green fluorescence proteins (GFP). The YFP (Topaz) Reporter Lentivirus contains replication-defective, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain an EF1α promoter-driven YFP(Topaz) construct (a.a. sequence below), and confer both YFP (Topaz) expression and puromycin resistance to the target cells (Figure 1). YFP (Topaz) expression and transduction efficiency can easily be verified and optimized via fluorescence microscopy or flow cytometry. Topaz has an excitation wavelength of 514 nm, an emission wavelength of 527 nm, and extinction coefficient of 94,500$M^{-1}cm^{-1}$.

Application
- Optimizing transduction assays
- Easily track transduction efficiency over time
- Generation of YFP (Topaz) Reporter stable cell lines

Reference
Cubitt, A.B. *et. al*., Understanding structure-function relationship in the *Aequorea victoria* green fluorescence protein, *Methods in Cell Biology*, 1998;**58:**19-30.

Formulation
The lentiviruses were produced from HEK293T cells in 90% DMEM medium containing 10% FBS.

Titer
Two vials (2 x 500 µl) of YFP (Topaz) reporter lentivirus at a titer ≥5 x 10^6 TU/ml. The titer will vary with each lot, and multiplicity of infection will depend on the target cell type. We provide a standardized p24 measurement with each shipment.

Storage
Lentiviruses are shipped on dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal.

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Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

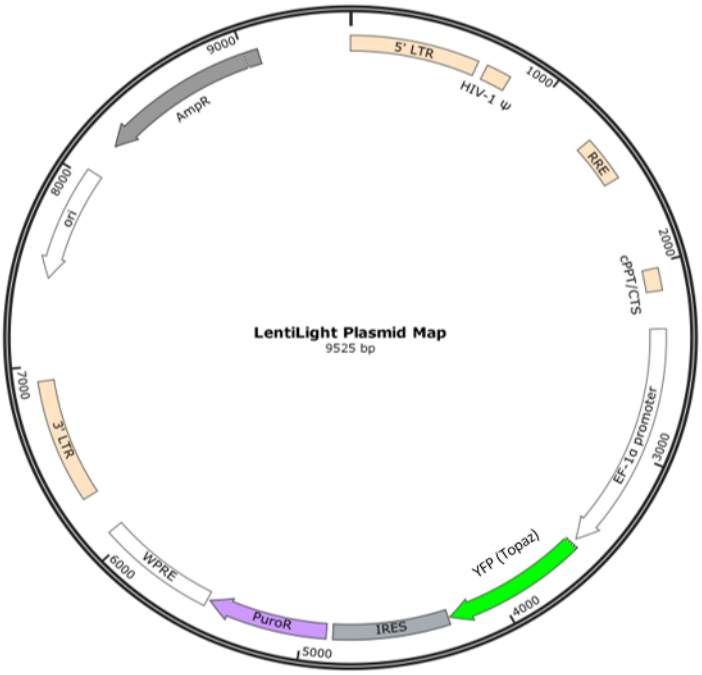

Figure 1. Schematic of the lentiviral vector used to generate the YFP (Topaz) reporter.

Materials Required but Not Supplied

- Thaw Medium 10 (BPS Bioscience #79704):
  RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1 mM Sodium pyruvate (Corning, #25-000-CL), 1% Non-essential amino acids (Corning, #25-025-CL), 1% Penicillin/streptomycin (Thermo Fisher, #15140122)
- Recombinant Human IL-2 (BPS Bioscience, #90184)
- Polybrene (Millipore, #TR-1003-G)
- Puromycin (Invivogen, #ANT-PR-1)
- T cell activation reagents (αCD3/αCD28 or Phytohaemagglutinin (PHA))
- Recombinant Human IL-17 (BPS Bioscience, #91014) (optional)
- Recombinant Human IL-15 (BPS Bioscience, #90180) (optional)
- 6-well and T75 tissue culture-treated assay plates
- Flow cytometer or fluorescence microscope

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Assay Protocol

The following protocol is a general guideline for transducing primary human T cells using the YFP (Topaz) reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be determined according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, such as primary T cells, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to performing downstream assays.

1. Day 1 (for frozen cells; skip to Day 2 if starting from fresh cells): Thaw T cells by lightly agitating frozen cells in a 37°C water bath until less than 20% is still frozen (about 2-3 minutes for a 1-2 ml aliquot). Spray thawing tube(s) with ethanol and wipe clean, then allow any remaining thawing to occur over ice. Once thawed, gently transfer cells to a 50 ml conical vial. Next, add 10 ml pre-warmed 37°C Thaw Medium 10 dropwise while swirling the 50 ml vial. Centrifuge at 300 x g for 10 minutes, and aspirate media. Break up cell clumps by flicking the bottom of the tube, then resuspend cells at 1 x 10^6 cells/ml Thaw Medium 10 plus 2 ng/ml IL-2. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: Add the desired T cell activation reagents (we recommend αCD3/αCD28 stimulation). Incubate the plate at 37°C with 5% CO_2 for 24-48 hours.

3. Day 4: Thaw YFP (Topaz) Reporter Lentivirus as described for T cells in Day 1. Centrifuge T cells and resuspend them in fresh Thaw Medium 10 plus 5 µg/ml polybrene. Calculate the desired titer/MOI and add thawed virus to cells*. We recommend performing the transduction in a small volume tube, at a cell density of at least 10 million/ml. Incubate the tubes at 37°C with 5% CO_2 for 6-18 hours.

4. Day 4-5: During the last 2 hours of infection, spin the cells/virus at 800 x g for 2 hours. Replace any media still containing virus or polybrene within 18 hours from the start of infection, to minimize T cell toxicity. The infection media should be replaced with pre-warmed Thaw Medium 10 plus IL-2 (10 ng/ml). Depending on your desired T cell subtypes, we recommend also adding IL-7 and IL-15 (2 ng/ml each), to support the survival and proliferation of all memory subtypes. Incubate at 37°C with 5% CO_2 for at least 72 hours.

5. Days 8-13: Check YFP(Topaz) expression with a fluorescence microscope or flow cytometer before selecting for puromycin resistance with 0.5 µg/ml puromycin. YFP(Topaz) expression should begin to appear at ~72 hours. If YFP (Topaz) expression is still too low, another round of infection can be done by repeating the procedure from step 3.

6. Maintain transduced cells in fresh Thaw Medium 10 and homeostatic cytokines as necessary in T75 flasks. Many dead cells from puromycin will be present at first, but these

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can be diluted via centrifugation or removed with a dead cell removal kit. Another round of T cell activation can help restore higher numbers of live, transduced cells.

Important Notes:
**Tip: Transduction efficiency can vary between lentivirus lots and different cell donors. While using a higher MOI/titer can provide limited improvement, we recommend optimizing this step by minimizing the overall infection volume and maximizing T cell density.*

The following Lentiviral Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

1) Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.

2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

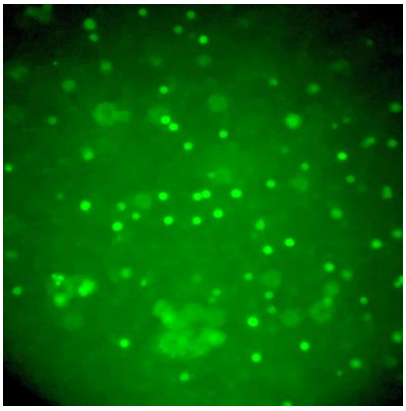

Figure 2. YFP (Topaz) expression in YFP (Topaz) Reporter Lentivirus transduced, primary pan T cells. 10 million activated T cells were resuspended in 500 µl Thaw Medium 10 and infected with 375 µl YFP (Topaz) reporter lentivirus (~50 million TU) for 6 hours as described above (last 2 hours at 800 x g). Photo taken with a fluorescence microscope (40X objective) one week after transduction.

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Sequence (variation of mVENUS, GenBank #AUG68261,
with L69V and K80R mutations)

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTT
FGYG**V**QCFARYPDHM**R**QHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELK
GIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGD
GPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

Related Products

Product	**Cat. #**	**Size**
GFP Reporter Lentivirus	79703	500 µl x2
NFAT/eGFP Reporter Lentivirus	79922	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Reporter (Luc) - A549 Cell Line	60625	2 vials
NF-κB Reporter (Luc) - HCT116 Cell Line	60623	2 vials
NF-κB Reporter (Luc) - CHO-K1 Cell Line	60622	2 vials
NF-κB Reporter (Luc) - Jurkat Cell Line	60651	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml