



# YFP iPS Cell Line

iPS Cell Line with LanYFP reporter

In order to save time and eliminate the need to generate iPSCs for experimentation, Allele Biotech offers ready to use iPS cell lines. This particular iPS cell line was generated using Allele's own NextGen mRNA reprogramming technology. The LanYFP transgene was integrated using lentivirus, allowing the cells to fluoresce yellow using proper filter sets.

## Reprogramming

There are several methods currently employed to generate "footprint-free" iPSCs: episomal DNA, Sendai virus, protein transduction, and messenger RNA (mRNA). Of these various methods mRNA has emerged as a logical choice as it allows for real time expression control, is completely DNA free and eliminates cleanup steps. We have striven to eliminate common difficulties with this method by creating new reagents and developing a stream lined feeder-free protocol that slashes the hands-on time required for reprogramming. This next generation mRNA reprogramming technology established by Allele allows for the high efficiency generation of footprint free iPSC with out use of feeder cells and xeno-contaminated reagents. iPS cellines provided by Allele were generated using this new protocol ensuring researchers high quality and healthy pluripotent cells for the potential downstream application of choice.

## YFP Expression

This particular cell line expresses LanYFP, one of the brightest fluorescent proteins (~3 fold brighter than EGFP). The LanYFP transgene is introduced to iPSC's via lentivirus. Expression of LanYFP allows for tracking and live imaging of iPS cells.

## Features

- ◆ Reprogrammed using Allele's Next Gen mRNA technology
- ◆ Pluripotency Verified; validation at the molecular level by qRT-PCR and antibody staining for nuclear and cell surface markers
- ◆ Ready to use cell aliquots
- ◆ Fully documented cell types and cell origins
- ◆ Unrestricted use for research (Non-academic and for profit companies as well as commercial usage require additional licensing fees)
- ◆ Expression of LanYFP reporter

### Box 1 | Product Info

Cat#: ABP-SC-YFPIPS	
Cell Type	Human iPSC (Induced Pluripotent Stem Cells)
Reporter Gene	Lan YFP Ex: 513, Em: 524, QY: 0.95, ε: 125,000
Source	Human Dermal Fibroblasts-Adult [HDF-a]
Qty	10 <sup>6</sup> cells in 1 vial

### Box 3 | Thaw Recommendation

#### Tissue Culture Plate preparation:

1. Coat 1 well with CELLstart (Invitrogen) according to protocol.
2. Replace CELLstart with 3ml MEF-conditioned media (R&D Systems) + 10uM Y27G32. Equilibrate media in regular incubator at 37°C. Then add 10 ng/ml bFGF.

#### Cell Thawing and plating:

1. Remove vial of iPSCs from liquid nitrogen storage. Quickly place vial in a 37°C water bath without submerging the top.
2. Remove the vial from the water bath just before it is fully thawed (a small ice fragment may remain). Spray the vial with 70% ethanol and place it in a cell culture hood.
3. Pipet the cells from the vial gently into a sterile 15-mL centrifuge tube using a sterile serological pipette.
4. Cautiously add 1 mL of warmed conditioned medium dropwise to the cells in the 15-mL centrifuge tube. While adding the medium, gently move the tube back and forth to mix the cells. Adding the medium slowly prevents osmotic shock to the cells.
5. Centrifuge the cells at 100 rcf for 5 minutes at RT.
6. Aspirate supernatant.
7. Gently bring up cell pellet in media from the well (from Step 2 of Tissue culture plate preparation) by gently pipetting up and down, then transfer back to the well.
8. Replace media after 24 hours (with bFGF and without Y27632)