Protocol for GFP immunofluorescence (whole Bacteria)

1. Inoculate a single colony in 1-2 ml of LB (+antibiotic) and incubate at 37 °C in shaker incubator overnight (starter culture)
2. The next day, dilute the starter culture in fresh LB (+antibiotic) to an OD_{600} of ~0.01 Add the required amount of IPTG for induction of protein expression and incubate the culture at 37 °C shaker.
3. Grow the culture until the OD_{600} reaches ~0.5- 0.6 (should take 3 h or so)
4. Spin down about 1 ml of the culture in an eppi, at 7-8K rpm, 4 °C, 5 min and carefully discard the supernatant.
5. Gently re-suspend the cell pellet in 1 ml of ice-cold 1X PBS, pH 7.4. Spin down the cells at 7-8K rpm, 4 °C, 5 min and carefully discard the supernatant.
6. Repeat step 5 (PBS wash) two more times.
7. **PFA fixation** (and mounting) can be done in two ways as given below:

a. Method based on Jose et al, 2005

i. Gently re-suspend the cell pellet in 0.25-0.5 ml of 1X PBS, pH 7.4
ii. Drop 30-50 µl of the resuspended cells onto a cover-slip
iii. Incubate at 37 °C until it is completely dry
iv. Transfer the coverslip (bacterial-side up) into 4 well or 24 well-plate
v. Add 0.5-1 ml of **4% PFA** (prepared in 1XPBS, pH 7.4; **see note below**)
vi. Incubate at RT for 20 min
vii. Remove the PFA solution and add 0.5-1 ml of 1X PBS, pH 7.4 and incubate at RT for 5 min. Repeat this wash step at least 3 times.
viii. Add a drop of mounting medium (Dako cytomation anti-fade mounting medium) on a glass slide and place the coverslip (bacterialside down). Allow to dry.

b. Quick (and dirty) method which I followed (and it worked)

i. After step 6, add 0.5 ml of **4% PFA** (prepared in 1XPBS, pH 7.4) to the cell pellet and very gently re-suspend by pipetting.
ii. Incubate at RT for 20 min
iii. Spin down the cells, 7-8K rpm, RT, 5 min and discard PFA solution
iv. To the pellet, add 0.5-1 ml of 1X PBS, pH 7.4, re-suspend by gentle pipetting, spin down the cells and discard supernatant. Repeat this wash step at least 3 times.
v. To the washed pellet, directly add two to three drops of mounting medium (Dako cytomation anti-fade mounting medium)
vi. Very gently resuspend the cell pellet by pipetting (the medium is viscous)
vii. Add a drop of this cell suspension on to a glass slide and place a clean cover-slip over the suspension and allow to dry.

8. Proceed to imaging. Alternatively, the edges of the cover-slip can be sealed with nailpolish and slides can be stored at 4°C

Note:

1. PFA is very toxic! Perform all the steps involving PFA under the hood. Wear protective gloves etc.
2. **GFP fluorescence is pH-dependent!** Make sure that the pH of the 4% PFA solution is around pH 7.4 (see point 4 below).
3. Also, try to perform almost all the steps with eppis covered in Alu-foil to avoid bleaching.
4. **PFA preparation**. Add 4 grams of PFA to approximately 70mL of water. Heat up to 60 degrees in a water bath (do not heat any higher). Add a few drops of 1M NaOH until the PFA goes into solution. Cool to room temperature and add 10mL of 10x PBS. Adjust the pH to 7.4 and bring final volume up to 100 ml.
5. **Blocking step**: Usually, for preparing mammalian cells for immunofluorescence, I perform a blocking step after PFA fixation, which I did not do in this quick protocol. Usually, quenching excess free and unreacted PFA is recommended and a good practice to avoid over-fixation as well as reducing auto-fluorescence.

a. After PFA fixation, remove the PFA solution
b. Add, 0.5 ml-1 ml of 50 mM Ammonium Chloride and incubate for 10 min
c. Remove the ammonium chloride solution and proceed with PBS wash

This protocol was kindly provided by Dr. Karthikeyan Radhakrishnan (Bielefeld University)

Reference:

Jose.J et al., (2005). Bacterial surface display library screening by target enzyme labeling: Identification of new human cathepsin G inhibitors. Anal Biochem. Volume 346, Issue 2, 15 November 2005, Pages 258-267 https://doi.org/10.1016/j.ab.2005.08.01