BIOTIN STAINING OF STREPTAVIDIN

To test if streptavidin is there, you can not only use an antibody, but also biotin that is fluorescently tagged directly.

MATERIALS
- Agar plate of your bacteria of interest, to pick colony
- Agar plate of the bacteria you want to use as a positive control
- Agar plate of the bacteria you want to use as a negative control
- 3 ml LB for overnight culture, and approximatively 5 ml to make the dilution in the morning
- PBS
- Eppendorf tubes
- Fluorescently tagged biotin (100x dilution of a stock solution of 50 mM)
- Microscopy slide and coverslip
- Cuvette to measure the OD
- IPTG

PROCEDURE
- Inoculate 1 colony in 3 ml LB containing the appropriate antibiotic
  
  Add 1mM IPTG if your protein is under the control of a lac promoter.

- Dilute the bacteria solution to an OD of approximatively 0.3
  
  With our bacteria, we had to put 1 ml of bacteria solution in 5 ml of fresh LB, but it depends how fast your bacteria grow.

- Pellet 500 µl of bacteria grown till OD 0.8
  
  We normally used an overnight culture, diluted it in the morning to an OD of 0.3 and then put it back in the incubator for approximatively 1 h to have an OD of 0.8

- Resuspend pellet in 200 µl PBS

- Add 4 µl of 100x diluted biotin

- Incubate 1 h on an eppendorf rotor
  
  covered with alufoil

- Wash 3 times with PBS
  
  To wash means to pellet the cells with a tabletop centrifuge, remove the supernatant and resuspend the pellet in 200 µl PBS.

- Pipett 2-10 µl on a slide and put coverslip on top, do microscopy
We advise you to let an advisor handle the microscope, since normally they are much faster in finding the right focus, which is important because the bacteria start dying on the slide.

To analyze images use a program as for example "image J" to make merge images