

Troubleshooting the immunofluorescence technique

Problem: Signal weak or missing; too few cells stained

Possible cause	Remedy
Protein is not expressed in cells	Prepare a cell extract and use Western blotting to verify that tagged protein is present in the cell.
Too few cells expressing protein	Use more cells in sample. OR Try a different transient transfection procedure or use a stably transfected cell line.
Poor cell permeability	Increase time of incubation with PBS-Triton (mammalian cells). Increase time of spheroplast formation (yeast cells).
Sample fixation before staining destroys epitope	Use a different sample fixation method.
Tag-specific antibody too dilute	Run a titration curve (identical samples, varying amounts of tag-specific antibody, optimal amounts of secondary antibody) to determine the optimal dilution of tag-specific antibody.
Wrong secondary antibody	Use a different secondary antibody.

Problem: High background

Possible cause	Remedy
Tag-specific antibody too concentrated	Run a titration curve (identical samples, varying amounts of tag-specific antibody, optimal amounts of secondary antibody) to determine the optimal dilution of tag-specific antibody.
Secondary antibody too concentrated	Run a titration curve (identical samples, optimal amount of tag-specific antibody, varying amounts of secondary antibody) to determine the optimal dilution of secondary antibody.
BSA in Blocking Solution contains IgG	Use only high purity (IgG-free) BSA in procedure.