

Staining Paraspeckles

Paraspeckles were discovered and characterized in the lab following the identification of the protein PSP1 (AF448795) in the nucleolar proteomics project. GFP fusions of this protein were observed to accumulate in speckles that are clearly distinct from the interchromatin granule clusters marked by antibodies to Sm and SR proteins, and this localization was confirmed for endogenous PSP1 with a polyclonal peptide antibody raised in rabbit. The peptide sequence used to generate the antibody is APPAPAPPEDHPDEEM, and the antibody is specific for human PSP1.

The peptide antibody works for both immunofluorescence (1:50) and Western blotting (1:2000 to 1:5000). One important consideration for cell staining is that the cells must be paraformaldehyde fixed, and for no longer than 5 minutes. We use 3.7% (w/v) paraformaldehyde in CSK buffer (10 mM PIPES pH 6.8; 10 mM NaCl; 300 mM sucrose; 3 mM MgCl₂; 2 mM EDTA) at room temperature.