Immunofluorescence Staining of PCNA and SV40 Tg
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1. Tissues were frozen in liquid nitrogen and stored at -70'c. 8um sections were cut on a cryostate and mounted onto polylysine coated slides and stored at -70'c.

2. Sections were taken out from -70'c and fixed in 1% paraformaldehyde for 60-90 seconds at RT when still wet. 1% paraformaldehyde were made by adding paraformaldehyde powder into TS buffer and heat to about 60'c, add small amount of NaOH and shake until dissolve, add 5N HCl to pH7, cool to RT before use.

3. Wash sections for 2x2min with TS, treat with 0'c 100% methanol for 10min, treat with 0.1% NP40 in TS for 5min, 0'c. Wash 3x3min with TS.

4. Block sections with 10% normal goat serum for 15min, remove blocking solution and add 1/100 dilution of mouse anti rabbit PCNA/cyclin monoclonal antibody(IgM,\(u\) heavy chain, K light chain) in 10% normal goat serum, RT 1hr. Wash 3x3min with TS.

5. Incubate with 1/100 dilution of FITC labelled goat anti mouse IgM(BMB Cat. 605 21) and IgG(BMB Cat. 605 240) in 10% normal goat serum for 1hr. Wash 3x3min with TS.

6. Incubate with Pab412 supernatant(10% normal goat serum added) for 1hr, wash 3x3min with TS. Incubate with 1/100 dilution of rhodamine labelled goat anti mouse IgG(BMB Cat. 605 140) in 10% normal goat serum for 1hr, wash 3x3min with TS. Mount slide with anti-fade.

Slides were observed using FITC and rhodamine filters and were photographed by double exposures. Normal exposure level using both filters will give good superimposed images. ASA400 Kodak color print films were used with CMU's departmental fluorescence scope. About 40 seconds were required for each exposure.

If only PCNA were needed to be stained, Pab412 staining can be omitted. In this case, 0.2ug/ml of propidium iodide can be added to the last 3min wash to help locate nuclei.

Pab416, Pab 419, Pab 113 can all give good staining of SV40 Tg using this short fixing protocol.

This protocol do not give good histology on immunohistochemistry staining. Nuclear fluorescence stain looks good.