IMMUNOFLUORESCENCE ON COVERSLIPS

(To be carried out on rounded cover slides or on cryostatic sections).

Important: Before carrying out the staining, the slide holder containing the cryostatic sections should be maintained for at least 1 hour at room temperature (with the cap closed) to allow defrosting without causing the crystallization of the section.

• Wash in PBS and proceed with fixation which can be performed with two different methods (depending on the antibody used):

A) Incubation with Methanol for 10 minutes at -20°C and then wash in PBS;

or

B) Incubation with 2% Paraformaldehyde for 20 minutes at 37°C.

• Wash with PBS twice for 5 min and leave in the refrigerator at 4°C if staining is carried out later.

• Permeabilize with PBT for 10 minutes 3 minutes on ice (PBT = PBS + 0.1% Triton-X100).

• Wash in PBS.

• Add the primary antibody for 1 hour at 37°C. The primary antibody must be diluted in PBS + 0.05% Tween-20 + 3% BSA (for 50 ml of PBS add 1.5g of BSA and 25µl of Tween-20).

• Wash with PBS twice for 5 minutes.

• Incubate the secondary antibody with the same conditions as the primary. To color the DNA with Toto3, add RNAse in this step at diluted concentration of 1:50.

• Wash in PBS.

• Add Toto3 (diluted 1: 1000 in PBS) and incubate for 10 minutes at room temperature.

• Wash in PBS.

• Mount and then observe.

Autor: M. Todaro **01/02/2015** **Approved: Giorgio Stassi**