Protocol example for indirect immunofluorescence labelling of peptide epitopes in adhering cells

- Grow cells on suitable coverslips (thickness 1.5).

- Remove medium from cells and wash 2x with PBS (37°C). It is important to remove all unwanted debris and dead cells from the sample.

- Fix cells with 4% para-formaldehyde (fresh in PBS) for 30' at 4°C (this step can be extended if necessary).

- Remove para-formaldehyde from cells and wash 3x with PBS.

- Add 50 mM NH_4Cl in PBS and incubate for 10'. Alternatively a freshly made solution of 1 mg/ml sodium borohydride in PBS can be used.

- Remove NH_4Cl (or $NaBH_4$) and wash 2x with PBS

- Permeabilise samples with 0.1 - 0.5% Triton X-100 in PBS for 15'

- Remove solution and wash 2x with PBS

- Add block solution (see below) and incubate for at least 30' to block low-affinity, unspecific protein binding.

- Remove block solution and add primary antibody in block solution referring to the antibody specifications. Incubate for at least 1h at RT (O/N optional) at very gentle movement.

- Remove primary antibody and wash at least 3x with wash solution (see below) for a minimum period of 45'.

- Add secondary antibody conjugate in block solution and incubate for at least 1h at RT (O/N optional) at very gentle movement. From this step onwards, protect samples from light.

- Remove secondary antibody and wash at least 3x with wash solution for a minimum period of 45'.

- Wash with PBS for 10' to remove the detergent.

- Drain the PBS well and air-dry sample to avoid refractive index mismatches in your mounting medium. Apply an appropriate amount of mounting medium to a clean slide/coverslip and carefully assemble 'glass sandwich' whilst avoiding to generate air bubbles in the medium.

- Leave mounting medium to cure (refer to supplier's information for duration). Note that for many mountants the refractive index of the medium changes significantly over the first 48 hours.

NOTE: If you use fluorescent proteins or other acetone-sensitive components in your experiment NEVER use nail varnish to seal the coverslip!

Block solution	**Wash solution**
10% FCS 0.2% Tween-20 in PBS	1% BSA (w/v), optional 0.2% Tween-20 in PBS

This protocol might not be comprehensive and we take no responsibility for its content. It should serve as a guideline and optimised for individual experiments.