Multiple labeling at single cell level

There are many situations, which need to study minimally two markers expression. For example, the expression of some marker in tumor should be studied. Sometimes, it is not easy to distinguish the tumor cells from the stromal elements. Using the technology of multiple (frequently double) labeling, the tumor cells can be marked by the antibody (for example anti-pancytokeratin) and the expression of the second marker can be precisely studied in the defined tumor cell population. For the immunocytochemistry, both used the first-step antibodies must be produced by different animal species (mouse vs. rabbit or goat). These antibodies can be subsequently detected by the labeled species-specific second-step antibodies.

Enzyme labeled second-step reagent

The peroxidase or alkaline phosphatase is frequently used as labels of second-step reagents for this purpose. The permanent specimen, that can be archived for almost non-limited time, can be received using this system. This type of specimen can be easily counterstained, which improves the orientation in the section. Moreover, the essential light microscope can be used for the specimen evaluation without any additional equipment. On the other hand, the endogenous enzymatic activity (according to used marker) must be blocked before the reaction because of the risk of false positive results. This blocking can be in distinct cell populations quite problematic.

Fluorescent marker labeled second-step reagent

The main advantage of this system is the exclusion of the blocking of endogenous enzymatic activities of cells and tissues. On the other hand, the specimens cannot be stored for a long time. Although, the specimens can be counterstained to (for example DAPI staining of nuclei), the orientation in the section is not so easy. Moreover, the use of optical microscope equipped with special UV light source, filters and objectives is necessary. The bleaching of fluorescence signal during to the UV light exposition is also problematic, although, special mounting media can reduce this phenomenon.

The precise topical localization of distinct molecules in cells and extracellular matrix represents an important topic of the cell molecular biology, immunology and pathology. The immunohistochemical, lectin-histochemical and in situ hybridization procedures are employed for this purpose. The selection of convenient detection system represents a crucial step of experimental procedure.

Simple labeling

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Enzyme labeled second step reagents

In case of enzyme labeled reagents (alkaline phosphatase, peroxidase), the presence of positive reaction is marked usually with brown (peroxidase) and blue (alkaline phosphatase) signal. In case of extensive reaction against highly expressed antigens, the signals can be mutually masked and the precise colocalization (for example both antibodies are directed against different molecules located in the same organelle) is problematic and false results are frequently received.

The fluorescence labeled second step reagents

The most frequently used labels are the derivatives of fluorescein isothiocyanate (green signal) and teramethylrhodamine isothiocyanate (red signal). Moreover, both labels are excited by different wavelength of light, which enables to observe the result of double labeling of biological specimen separately using the specific filters. The scale of labels and specific filters commercially available is extremely wide and the most suitable combinations can be selected. Although in the classical fluorescence microscopy each signal is detected separately, the specific double (triple) band filters are available and they permit the simultaneous detection of both signals. However, these special filters are fastidious for light intensity and the photo documentation is also not so precise in comparison with the use of mono band filters separately.
Employment of LUCIA system in the fluorescence multiple (double) labeling technology

Our LUCIA system works with digitized figures harvested with CCD monochromatic integrating TV camera (COHU). The used system is highly sensitive, which enables very short exposition time. In combination with anti-bleaching mounting media, the UV bleaching of specimens is almost excluded. The distinct separate signals (green, red, blue) can be combined to one merged figure. LUCIA is, therefore, highly recommended for the colocalization experiments at the single cell level. The presence of two signals (green, red) in the same cell structure can be well recognized according to the color shift of merged signal to yellow. For example, the colocalization of binding sites for galectin-3 (red signal) with desmosomal protein desmoglein (green signal) was shown by yellow color. This result can be interpreted by the association of galectin-3-reactive glycoligands with the cell surface, because it is the typical position of desmoglein expression (Fig. 1). The third canal (blue) can be used for the counterstaining of cell nuclei (DAPI) (not shown). The function of the measurements of fluorescence profile is very suitable for support of colocalization. The same position of peaks of measured signals in the x-axis indicates (in combination with microscopic feature) the precise colocalization at the cellular level (Fig. 2). Moreover, this mode can be employed in the semiquantitative measurements of the fluorescence signal intensity. The figures of each separate signal or the merged figures are of a high quality and in combination with the sublimation or ink printer the prints are of the publication quality. The preparation of figures, such as the combination of several pictures to one print is easy and comfortable. The LUCIA system is highly compatible, which is important for the electronic transmission of figures. This is highly evaluated, namely, in course of long-distance collaboration.

According to our personal experience, LUCIA is highly effective equipment for multiple labeling technology in histology, cell biology, pathology and immunology. The results are comparative in many cases with confocal laser scanning microscopy, although the price of fluorescence microscope equipped with this analysis system represents the fragment of price of confocal system.

REFERENCES:


