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Tyramid amplification system, an old and unappreciated powerful immunohistochemistry problem solving weapon. Hints for a simple and easy use of it

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The tyramide amplification system was first used in immunohistochemistry in the decade of 1990, but has never been very popular in spite of its capacity to increase in an extraordinary way the sensitivity and specificity of the regular immunostaining. The tyramide principle of action is based on the reactivity of this molecule with horseradish peroxidase (HRP) enzyme which causes a catalytic reporter deposit in close vicinity to the epitope of interest. With this system we are able to use primary antibodies at a very high dilution which makes our immunolabeling more specific and also less expensive. It is also capable of detecting molecules that are very scarce or small or the ones that give weak signaling in our tissue, as it highly increases the size and intensity of the signal, enabling us to visualize a very precise and enhanced label without loss of resolution or increase in background. Other advantage of this amplification system includes the possibility to do a dual immuno-labeling with primary antibodies made in the same species, like the use of two primaries from mouse. Regarding the protocol of use of this technique, it is very important to utilize specific washing and protein blocking buffers to prevent having background or non specific binding. To do the tyramid system more versatile is recommendable to have HRP conjugated streptavidin (SA-HRP) which would favor the use of secondary biotinylated antibodies and to have HRP conjugated anti-fluorescein, that would allow to continue with the amplification step of an already fluorescein labeled slide with a weak signal. Finally the anti-fluorescein-HRP could be used with a fluorescein conjugated tyramid reagent to do a chromogenic detection without the need of a fluorescence microscope.

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