Optimization of cancer cell detection using multiplex tyramide signal amplification.

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Description:

Classic immunohistochemistry (IHC) assays can typically assess expression of one or two proteins per tissue section. We have developed a novel immunofluorescence staining protocol to detect a panel of seven proteins on PCa tissue from primary tumor biopsies and metastatic lesion autopsy tissue, as well as cancer cells from liquid biopsies. We used a tyramide-based system to amplify the true signal and optimized the protocol to reduce background signal,
thereby boosting the signal-to-noise ratio. Any protein-specific antibody in this protocol can be exchanged for a different validated antibody. This protocol therefore, represents a highly informative and flexible assay that can be used to provide important information about cancer tissue for the purpose of improving detection, diagnosis, and treatment.

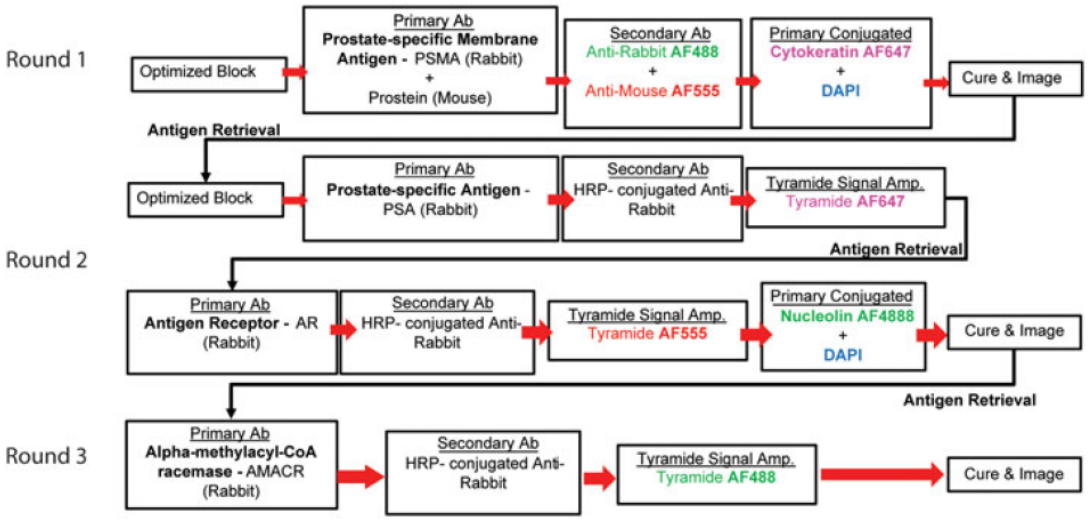

FIGURE 1 Novel multiplex tyramide signal amplification immunofluorescence protocol. Multiplex tyramide signal amplification can be organized in a flexible protocol divided into three rounds of staining separated by heat-induced antigen retrieval and imaging. Optimized block includes a combination of TrueBlack, Image-iT FX Signal Enhancer, and 5% BSA. Anti-human primary antibodies can be substituted for mouse-specific or other species-specific antibodies without requiring alterations to subsequent secondary antibody or tyramide reagents. Curing is an overnight process and imaging is followed by overnight coverslip removal before the subsequent antigen retrieval and blocking steps can begin. Round 2 contains an antigen retrieval step to allow for the usage of two primary antibodies both made in rabbit. Any of the three rounds may combine primary antibodies of the same species given that they are separated by an antigen retrieval step and are paired to different tyramide fluorophores. It is important to note that once a specific tyramide fluorophore has been used that channel may no longer be used in following rounds. BSA, bovine serum albumin; DAPI, 4′,6-diamidino-2-phenylindole; HRP, horseradish peroxidase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen

Publications:

Roy S, Axelrod HD, Valkenburg KC, Amend S, Pienta KJ. Optimization of prostate cancer cell detection using multiplex tyramide signal amplification. J Cell Biochem. 2018 Nov 2. PMID: 30390333

Axelrod HD, Pienta KJ, Valkenburg KC. Optimization of Immunofluorescent Detection of Bone Marrow Disseminated Tumor Cells. Biol Proced Online. 2018 Jul 1;20:13. PMID: 29988526