Multiplex Immunostaining -Simultaneous Vs. Sequential



Multiplex Immunostaining - Simultaneous Vs. Sequential

Many automated immunohistochemistry (IHC) instruments provide an option to perform multiplex immunostaining, also known as multiplexing. Multiplexing is used to identify the presence of multiple biological markers on a single tissue sample, allowing pathologist to analyze several targets concurrently to aid in diagnosis. Not all multiplexing, however, is created equal.

Certain IHC instruments today only permit sequential staining methods. Sequential procedures were the first to be developed and consist of multiple antigens being labeled one after another in "sequence." When compared to simultaneous procedures, the sequential staining process requires substantially more time, usually 40-60 additional minutes per target-biomarker. A sample procedure for sequential staining is shown below:



Other IHC instruments, such as Biocare's intelliPATH FLX and IQ3000, accommodate sequential and simultaneous staining, while the ONCORE Pro only accommodates simultaneous multiplex staining. Simultaneous staining typically uses cocktailed primary antibodies and detection systems, which significantly reduces protocol steps and overall turn-around time. The primary antibody cocktails are comprised of different animal species, differentiating the target-biomarkers. A typical protocol for simultaneous staining consists of an epitope retrieval, one primary antibody cocktail incubation, one cocktailed secondary antibody incubation, 1st chromogen incubation, followed by the 2nd chromogen incubation.



For comparison, a routine prostate multiplex IHC stain performed sequentially on the Ventana Benchmark Ultra takes on average at least 2 hours longer than when performed simultaneously on the Biocare intelliPATH FLX.¹ When working against the clock, this saving of time, along with patient tissue, proves most beneficial.

Interested in saving your lab time and tissue? Biocare offers an array of cocktailed primary antibodies and multiplex detection systems that may help achieve these goals. Available in prediluted formats, these cocktailed primary and secondary antibodies can be used both manually and on most automated IHC instrumentation. For more information, please call 1-800-799-9499 or visit our website: https://biocare.net/product-category/multiplex-ihc-antibodies/ (for antibodies) https://biocare.net/products/multiplex-detection/ (for detection systems)

1. Myers J. (2018, May) Chromogenic Multiplex Immunostaining: A Clinically Useful Procedure That Hasn't "Caught On" (and Why). Presented at The 2018 Tri-State Histology Symposium, Maddison, WI. http://www.whs.wildapricot. org/resources/Documents/Chromogenic%20Multiplex%20Immuno-Staining%20Joe%20Myers.pdf www.biocare.net

Biocare Medical Mutliplex



CK 5/14 + p63 + CK 7/18 (left and middle) and CK HMW + p63 + AMACR (right) great examples of Biocare's antibody cocktails and use of multiplex detection

Biocare Medical IHC Instrumentation Multiplex Capabilities

Instrument	Simultaneous Multiplex Ability	Sequential Multiplex Ability
intelliPATH FLX	\bigcirc	S
ONCORE Pro	\checkmark	8
IQ3000	\checkmark	