



If the laboratory uses an avidin-biotin complex (ABC) detection system (or a related system such as streptavidin-biotin or neutravidin-biotin), nonspecific false-positive staining from endogenous biotin is addressed.

NOTE: Biotin is a coenzyme present in mitochondria, and cells that have abundant mitochondria such as hepatocytes, kidney tubules and many tumors (particularly carcinomas) are rich in endogenous biotin. Biotin-rich intranuclear inclusions are also seen in gestational endometrium and in some tumors that form morules. If steps are not included in the immunostaining method to block endogenous biotin before applying the ABC detection complex, nonspecific false-positive staining may occur, particularly when using heat-induced epitope retrieval (which markedly increases the detectability of endogenous biotin). This artifact is often localized to tumor cells and may be easily misinterpreted as true immunoreactivity.

Blocking endogenous biotin involves incubating the slides with a solution of free avidin (which binds to endogenous biotin), followed by incubation with a biotin solution (which saturates any empty biotin-binding sites remaining on the avidin). Biotin-blocking steps should be performed immediately after epitope retrieval and before incubation with primary antibody.

REFERENCES

- 1) Miller RT, Kubier P. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of egg whites. *Appl Immunohistochem* 1997; 5: 63-66
- 2) Miller RT, Kubier P, Reynolds B, Henry T. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of skim milk as an economical and effective substitute for commercial biotin solutions. *Appl Immunohistochem & Molec Morphol* 1999;7:63-65
- 3) Allen M, Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.

ANP.22660 Control Slide Review

Phase II

The laboratory director or designee reviews batch control slides for acceptability before reporting results.

NOTE: Records of this daily review must be retained and clearly show that positive and negative controls for all antibodies stain appropriately. Batch control records must be retained for two years.

Immunohistochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the laboratory director following appropriate validation.

The batch control slides must be readily available to pathologists who are signing out cases. The location of the slides should be stated in the procedure manual.

Evidence of Compliance:

- ✓ Patient reports or worksheet with control results

REFERENCES

- 1) Shellhorn N. IHC troubleshooting tips. *Advance/Lab.* 2000;9(1):33-37
- 2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2023(Dec 28): [42CFR493.1273(f)].

****REVISED** 12/26/2024**

ANP.22750 Validation/Verification - Non-Predictive Antibody Marker Testing

Phase II



The laboratory has records of validation/verification of new antibodies, including introduction of a new clone, prior to use for patient diagnosis or treatment.

NOTE: The performance characteristics of each assay must be appropriately validated/verified before being placed into clinical use. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation/verification is at the discretion of the laboratory director and will vary with the antibody.