

| **Clone Wars – CDX2 – Comparative Analysis**

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Introduction

CDX2 is a nuclear transcription factor expressed in intestinal epithelial cells and commonly used as a biomarker in immunohistochemistry (IHC) to aid in the identification of colorectal adenocarcinomas and related tumors. Accurate and specific nuclear localization is essential for proper interpretation and diagnosis.

As part of our ongoing efforts to optimize assay performance and supply continuity, Biocare conducted a small comparative evaluation of three CDX2 antibody clones:

- CDX2 [CDX2-88]
- CDX2 [BC39]
- CDX2 [EP25]

This study was designed to provide laboratories with transparent guidance on clone selection. As always, laboratories and customers are encouraged to perform their own validation to provide optimal results tailored to the conditions within their laboratories.

Materials and Methods

A comparative evaluation was performed using formalin-fixed, paraffin-embedded (FFPE) tissue microarrays (TMAs) and additional tumor sections, including colon, ovarian, bladder, and melanoma tissues. All antibodies were evaluated under optimized IHC staining conditions on Biocare instrumentation using the same two-step detection chemistry. All tissues experienced Decloaking (or heat induced epitope retrieval) with citrate-based buffer. The CDX2 [CDX-88] and [EP25] clones mentioned in this assessment were sourced from different raw material suppliers, while the CDX2 [BC39] clone is maintained solely by Biocare Medical, LLC.

Results

1. Staining Performance on Colon Tissue Microarrays

- Both CDX2 [CDX2-88] and CDX2 [BC39] showed equivalent nuclear-positive case counts.
- CDX2 [BC39] demonstrated higher specificity, with clean nuclear staining and minimal background.
- CDX2 [CDX2-88] exhibited non-specific granular cytoplasmic staining overlapping nuclei in multiple tissues.

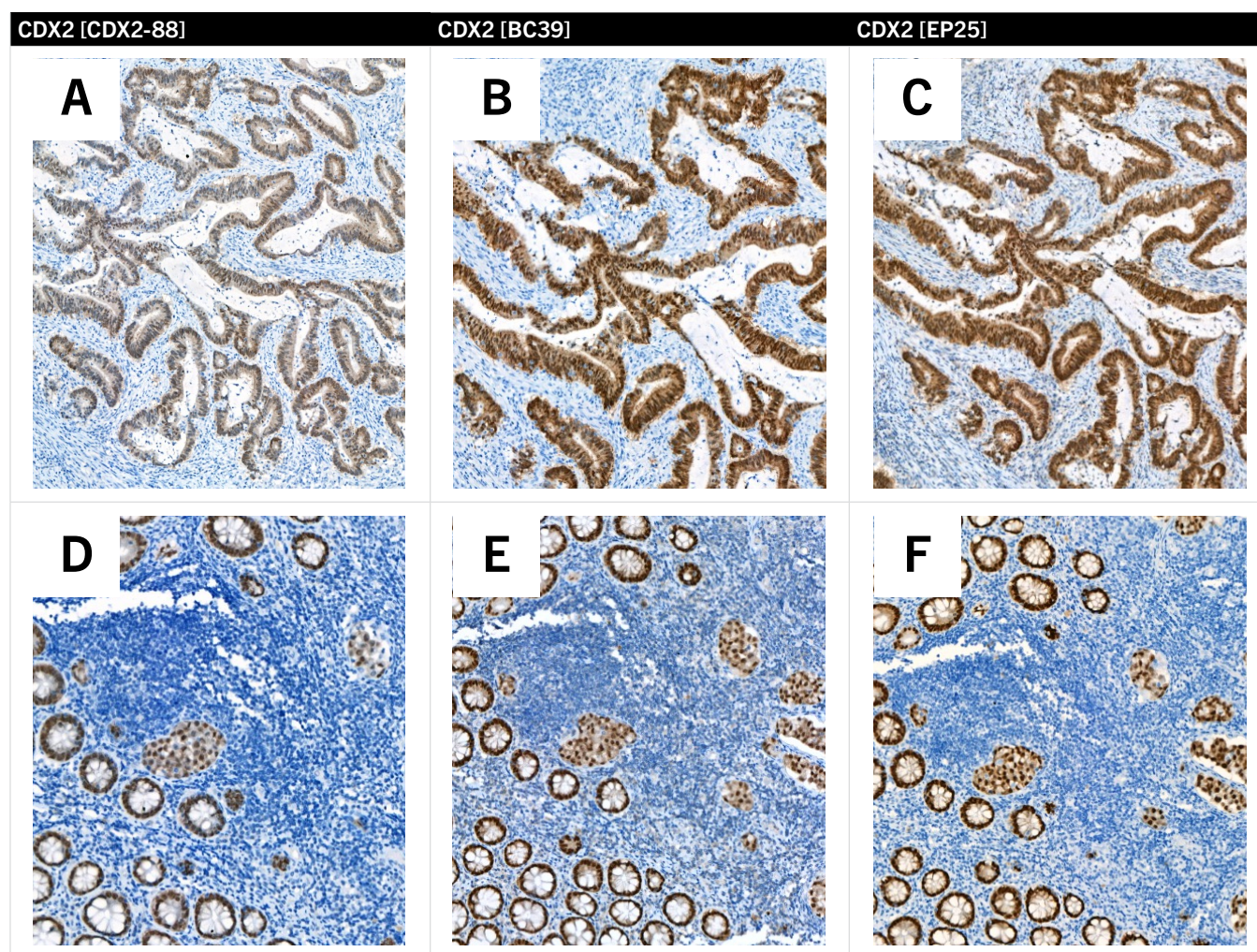


Figure 1. Comparison of CDX2 staining on Colon Cancer

Left (A, D): **CDX2 [CDX2-88]** — non-specific nuclear and cytoplasmic staining.

Middle (B, E): **CDX2 [BC39]** — clean, specific nuclear staining with clear lines of demarcation around non-CDX2 cells.

Right (C, F): **CDX2 [EP25]** — specific nuclear and cytoplasmic staining with minimal background staining

2. Cross-Tissue Evaluation

- To minimize bias and evaluate performance of other tissue types, bladder, ovarian, and melanoma tissues were also tested. CDX2 [BC39] maintained strong, specific nuclear staining and minimal cytoplasmic background.
- CDX2 [CDX2-88] again showed granular cytoplasmic staining, which could obscure nuclear interpretation.

These findings are based on internal comparative testing and are intended to provide performance guidance under standardized Biocare conditions.

Summary of Findings

Antibody	Species	Findings	Background	Overall Performance
CDX2 [CDX2-88]	Mouse monoclonal	Strong nuclear staining, but with non-specific cytoplasmic overlap	Moderate to high	Acceptable but artifact-prone with granular staining pattern
CDX2 [BC39]	Mouse monoclonal	Clean nuclear staining, equivalent sensitivity	Minimal	Superior specificity and recommended alternative based on internal evaluation
CDX2 [EP25]	Rabbit monoclonal	Strong, specific nuclear staining, with minimal background staining	Minimal to Moderate	Equivalent to mouse clones

Discussion

The evaluation shows that CDX2 [BC39] can provide equivalent nuclear sensitivity to CDX2 [CDX2-88], while offering improved specificity and reduced background staining. The elimination of cytoplasmic artifacts may enhance interpretability, particularly in tissues where non-specific staining could obscure true nuclear localization. For laboratories currently using CDX2 [CDX2-88], CDX2 [BC39] can represent a validated and seamless alternative, maintaining diagnostic continuity without compromising assay sensitivity or specificity.

While CDX2 [EP25] demonstrated strong performance as well, CDX2 [BC39] remains a robust, accessible, and reliable option, particularly for labs looking at alternative CDX2 clones.

Conclusion

This study demonstrates that CDX2 [BC39] can offer comparable nuclear sensitivity to CDX2 [CDX2-88] but with an improved in staining specificity and background cleanliness, based on internal comparative testing performed under standardized conditions. As pathology laboratories discover more antibody clones to highlight established biomarkers, the need for comparative analysis becomes more important. Clones like CDX2 [BC39] may provide ongoing consistency in CDX2 detection without compromising quality or workflow. By combining reliability with refined staining performance, CDX2 [BC39] may provide a confident path forward for accurate CDX2 assessment in daily practice. Laboratories are encouraged to perform verification under their site-specific protocols prior to adoption.