

Bond™ Oracle™ HER2 System – Interpretation of Staining on Gastric Cancer Tissue

- A hematoxylin and eosin (H&E) stained serial (paired) section of the tissue specimen should be available for reference to verify the presence of tumor.
- Only specimens from patients with stomach or gastroesophageal junction adenocarcinoma should be scored. In cases with intestinal metaplasia and gastric adenocarcinoma, only the gastric adenocarcinoma component should be scored.

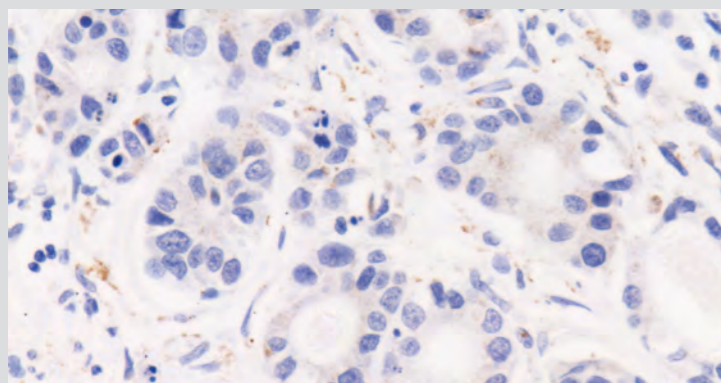
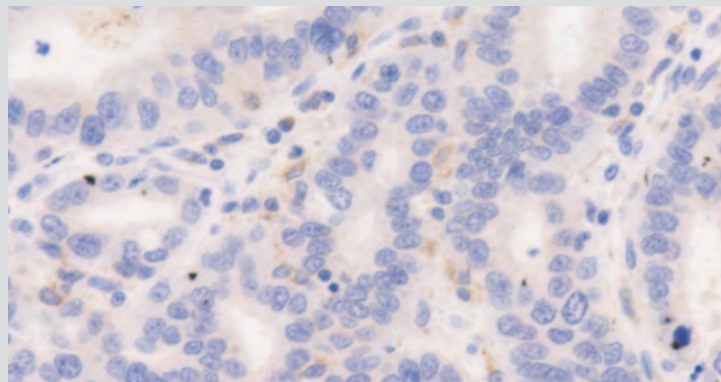
For each staining assessment, slides should be examined in the order presented below to determine the validity of the staining run and to enable semi-quantitative assessment of the staining intensity of the sample tissue.

1. HER2 Control Slide – validates assay performance
2. In-house positive control
3. In-house negative control
4. Patient tissue – HER2 Negative Control
5. Patient tissue – HER2 Primary Antibody

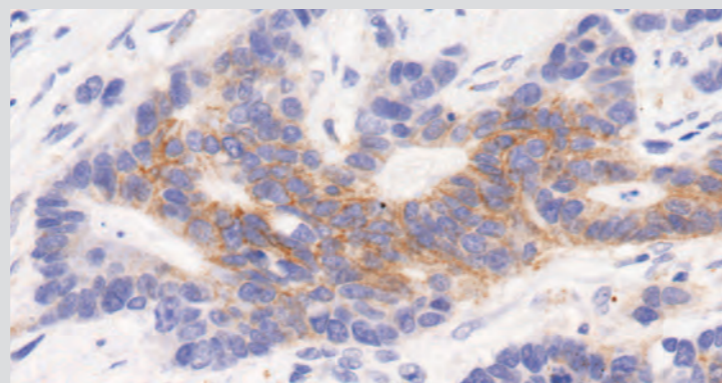
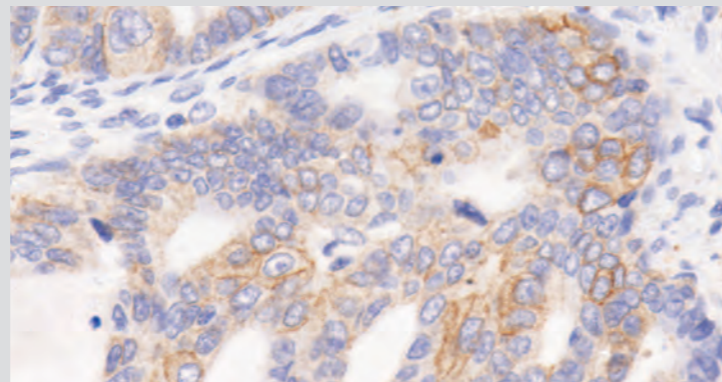
Surgical Specimens	Immunohistochemical Staining Pattern	Score	Assessment
	No staining is observed or membrane staining is observed in less than 10% of the tumor cells.	0	Negative
	Faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.	1+	Negative
	Weak to moderate complete, basolateral or lateral membrane staining is observed in equal to or more than 10% of the tumor cells.	2+	Equivocal (Weakly Positive)
	Strong complete, basolateral or lateral membrane staining is observed in more than 10% of the tumor cells.	3+	Strongly Positive

Biopsy Specimens	Immunohistochemical Staining Pattern	Score	Assessment
	No staining is observed in any tumor cell	0	Negative
	Tumor cell cluster with a faint/barely perceptible membrane staining is observed irrespective of percentage of cells stained.	1+	Negative
	Tumor cell cluster with weak to moderate complete, basolateral or lateral membrane staining is observed irrespective of percentage of cells stained.	2+	Equivocal (Weakly Positive)
	Tumor cell cluster with a strong complete, basolateral or lateral membrane staining is observed irrespective of percentage of cells stained.	3+	Strongly Positive

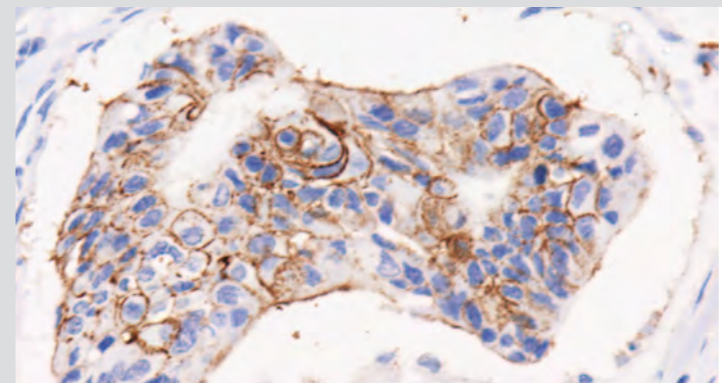
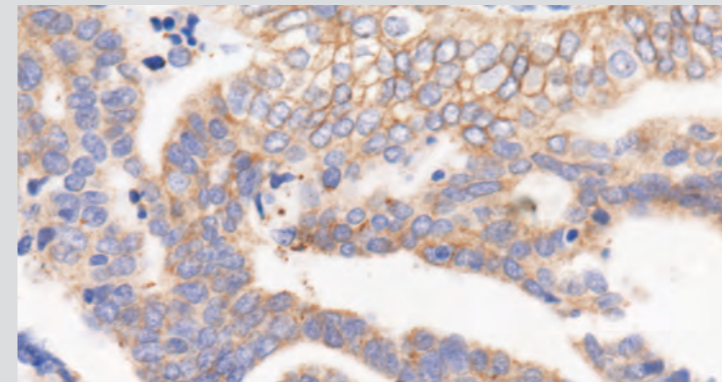
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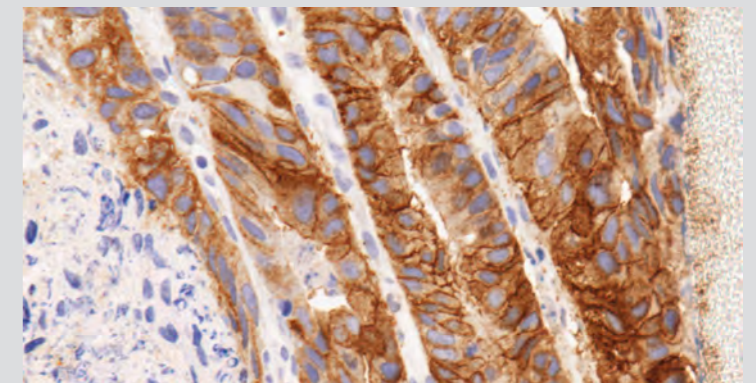
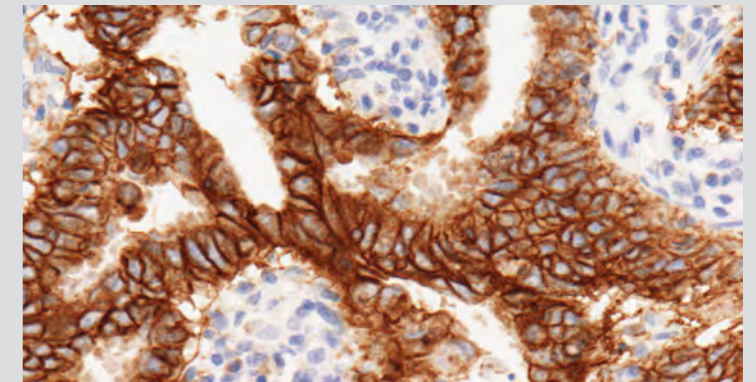
1+



2+



3+



HER2 Control Cell Lines

Why do we use control cell lines?

Oracle HER2 control cell lines are designed as assay quality controls. They ensure procedural accuracy of the Bond Oracle HER2 IHC System.

The Oracle HER2 control cells lines validate:

- reagent optimization and performance
- correct protocol implementation
- instrumentation performance

The Oracle 2+ cell line affords superior assay validation by representing the borderline 2+ expression level, the expression level most likely to be affected by any variation in an assay. Cell lines do NOT validate laboratory specimen preparation procedures or replace the requirement for appropriately fixed and processed in-house tissue controls.

A valid assay with the Oracle HER2 Control Slide shows the following:

0	1+	2+	3+
No staining in the 0 control cell line, MDA-MB-231	Presence of faint/barely perceptible brown, incomplete cell membrane staining in the 1+ control cell line, MDA-MB-175	Presence of weak to moderate brown, complete cell membrane staining in the 2+ control cell line, MDA-MB-453	Presence of strong brown, complete cell membrane staining in the 3+ control cell line, SK-BR-3

Important notes for evaluating the HER2 control cell lines

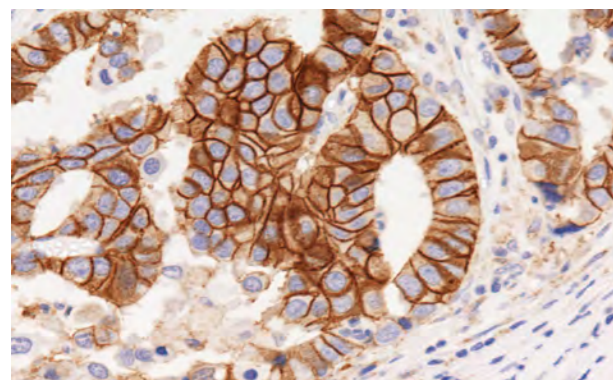
A feature of the MDA-MB-175 1+ control cell line is a distinct growth pattern in which the cells form clusters.

These clusters give rise to a continuous luminal brush border region across the cell cluster. This brush border staining will be stronger than that of the cell membrane and should not be included in the HER2 staining evaluation. It is the faint/barely perceptible incomplete cell membrane staining that is the correct HER2 oncoprotein 1+ staining pattern. Dot-like immunostaining of the Golgi region in the cytoplasm may also be observed in this cell line and should not be included in the HER2 staining evaluation. (For more information see Bond Oracle HER2 IHC System Interpretation Guide).

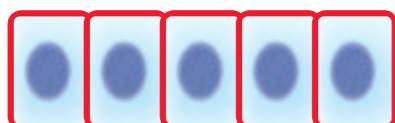
Minimal natural variation of the immunohistochemical profile will be seen between growth batches of cell lines utilized within the Bond Oracle HER2 IHC System. This natural variation is well within acceptable tolerance levels of a biological entity and does not affect the evaluation or performance of the system.

The HER2 control cell lines display consistent homogeneous staining as they are manufactured from a clonal population whose cells have a consistent gene/protein expression profile. When evaluating the HER2 control cell lines, the observer must be aware that the percentage rules applying to tissue (overleaf) do not apply to cell lines.

Tumor Profiling – Interpretable Components

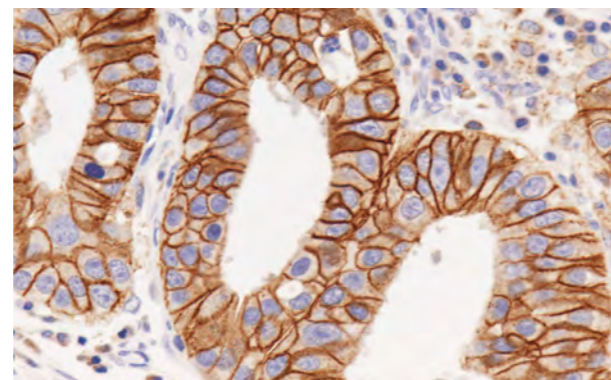


1. Complete Membrane Staining

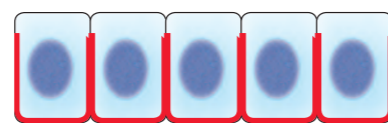


Complete Membrane Staining – IHC x40

Oracle HER2 IHC stained section illustrating complete membrane staining in gastric cancer

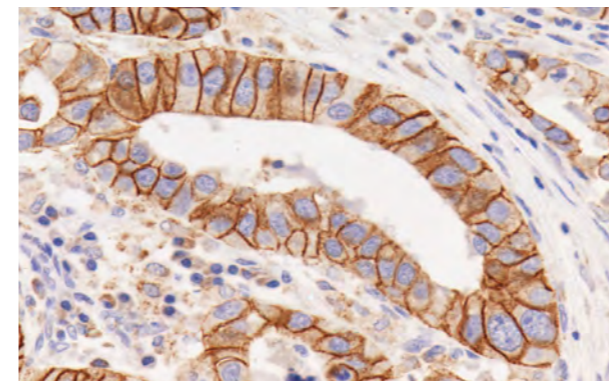


2. Basolateral Membrane Staining

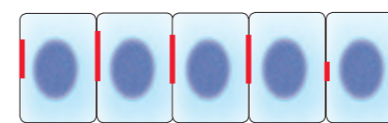


Basolateral Membrane Staining – IHC x40

Oracle HER2 IHC stained section illustrating basolateral staining in gastric cancer

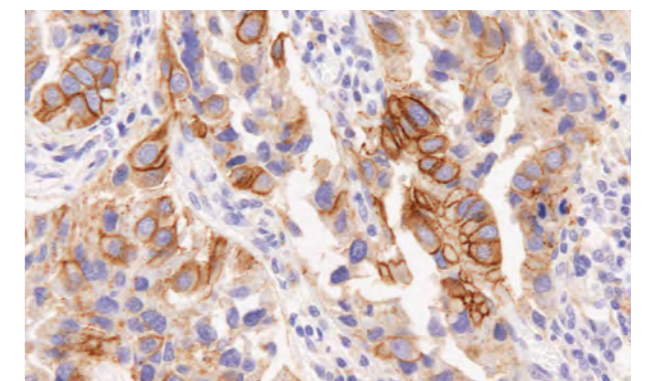


3. Lateral Membrane Staining



Lateral Membrane Staining – IHC x40

Oracle HER2 IHC stained section illustrating lateral staining in gastric cancer



4. Heterogeneous Staining

Lateral Staining – IHC x40

Oracle HER2 IHC stained gastric cancer illustrating areas of heterogeneous staining, with areas of mixed 3+ and 2+ staining