

## Interpretation of ONCOblot Test Results

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**MorNuCo, Inc. continues its monthly report for participating physicians and health professionals in order to answer common questions relating to the ONCOblot<sup>®</sup> Tissue of Origin (Cancer) Test.**

The ONCOblot<sup>®</sup> Tissue of Origin (Cancer) Test detects the presence of protein transcript variants of the ENOX2 cancer marker in blood serum. Based upon the charge (isoelectric point given as pH), size (molecular weight given in kilodaltons) and the number of ENOX2 protein transcript variants detected, the tissue of cancer origin can be identified.

### Number of Protein Transcript Variants

The number of ENOX2 protein transcript variants varies according to the tissue of cancer origin. For many tissues of origin (breast, lung, prostate, blood cell, and five others), only one ENOX2 protein transcript variant is produced. These ENOX2 proteins vary in average size and charge (Table 1), and can therefore be differentiated.

**Table 1.** Tissues of Cancer Origin that Produce One ENOX2 Protein Transcript Variant

<b>Cancer</b>	<b>MW (kDa)</b>	<b>pI (pH)</b>
Blood Cell	34-47	3.5-4.5
Breast	64-69	4.2-4.9
Cervical	90-100	4.2-5.4
Esophageal	42-47	4.6-5.2
Lung	52-56	4.1-5.3
Melanoma	37-41	4.6-5.3
Pancreatic	48-51	3.9-5.4
Prostate	71-88	5.1-6.5
Squamous Cell	57-68	5.0-5.4

For some cancers, two ENOX2 protein transcript variants are produced. These include: ovarian, hepatocellular, uterine, and six others (Table 2).

**Table 2.** Tissues of Cancer Origin that Produce Two ENOX2 Protein Transcript Variants

<b>Cancer</b>	<b>Protein 1</b>		<b>Protein 2</b>	
	<b>MW (kDa)</b>	<b>pI (pH)</b>	<b>MW (kDa)</b>	<b>pI (pH)</b>
Bladder	63-66	4.2-5.6	42-48	4.1-4.8
Hepatocellular	58-70	4.5-5.0	34-40	4.1-5.2
Mesothelioma	60-68	3.8-4.1	38-44	3.8-4.6
Ovarian	72-90	3.7-5.0	37-47	3.7-5.0
Sarcoma	50-55	5.2-5.6	37-45	4.3-4.9
Testicular Germ Cell	61-62	5.0-5.4	42-45	4.4-4.7
Thyroid Follicular	48-56	4.7-5.1	37-42	4.5-5.2
Thyroid Papillary	56-67	4.5-5.0	37-44	3.2-3.6
Uterine (Endometrial)	67-71	4.2-5.1	41-48	3.7-5.4
Uterine (Unspecified)	63-66	4.2-4.9	41-48	4.4-5.6

For three tissues of origin, three ENOX2 protein transcript variants are produced. These tissues of origin include: stomach, colon/rectum, and renal cell (Table 3).

The detection of four or more ENOX2 protein transcript variants may indicate cancer originating in the brain (Not shown).

**Table 3.** Tissues of Cancer Origin that Produce Three ENOX2 Protein Transcript Variants

<b>Cancer</b>	<b>Protein 1</b>		<b>Protein 2</b>		<b>Protein 3</b>	
	<b>MW (kDa)</b>	<b>pI (pH)</b>	<b>MW (kDa)</b>	<b>pI (pH)</b>	<b>MW (kDa)</b>	<b>pH (pH)</b>
Colorectal	80-96	4.4-5.4	50-65	4.2-5.3	33-46	3.8-5.2
Gastric (Stomach)	120-188	4.7-5.5	50-62	4.5-5.6	45-53	2.4-3.6
Renal Cell (Kidney)	69-73	4.7-5.4	54-61	4.1-5.2	38-43	3.7-4.3

The number of transcript variants is primarily an indication of the tissue of cancer origin and has no apparent relationship to disease severity, progression or resistance to treatment.

For tissues of origin that produce multiple ENOX2 protein transcript variants (Tables 2 and 3), it is possible for one or two ENOX2 proteins to be either absent or produced below the lower limit of detection of the assay. This occurs in approximately 3% of ONCOblot<sup>®</sup> tests of clinically-confirmed cancer patients (1).

#### **Molecular Weight**

The size of each ENOX2 protein produced by a cancer of any given tissue of origin fall within the ranges indicated above. For any given cancer, the size of each ENOX2 protein is predicted to remain within this range for the duration of the disease. An average variation of  $\pm 2$  kilodaltons is expected as the upper and lower limit of accuracy in molecular weight measurements. The molecular weight provides information regarding the tissue of origin and is unrelated to the disease stage, progression or severity.

#### **Isoelectric Point**

The charge of an ENOX2 protein transcript variant is measured with an average variation of  $\pm 0.2$  pH units and falls within the ranges indicated above for each tissue of origin. The isoelectric points of ENOX2 proteins have not been shown to change during the course of the disease, and these values are unrelated to disease stage, progression or severity.

#### **Absence of ENOX2 Transcript Variants**

The absence of ENOX2 protein transcript variants within an ONCOblot<sup>®</sup> Test indicates that ENOX2 proteins are either not present or produced at concentrations below the lower limit of detection of the test. The current limit of detection of ENOX2 is estimated to be produced by as few as 2 million cancer cells, equivalent to a solid tumor between 0.8 and 1.2 mm in diameter (2).

#### **Summary**

The ONCOblot<sup>®</sup> Tissue of Origin Test confirms cancer presence based upon the detection of one or more ENOX2 transcript variants in a subject's blood serum. Depending on the number of transcript variants, their size and their charge, the tissue of cancer origin can be determined. However, no additional information as to stage, progression, or disease severity can be derived from these values.

#### **References**

1. Morr  DJ, Gilmartin D. Estimation of the Accuracy of the ONCOblot<sup>®</sup> Tissue of Origin Cancer Test. ONCOblot<sup>®</sup> Reports 1 (No. 1), May 2015.
2. Taggart DJ, Morr  DJ. Estimation of the Lower Limit of Detection of ENOX2 in Serum. ONCOblot<sup>®</sup> Reports 1 (No. 5), September 2015.