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## **Gastrointestinal Tumor Antigen (Ca19-9)**

This large glycoprotein is defined by the carbohydrate epitope Ca19-9 located on MUC-1, and is referred to as sialylated lewis A (Lea). It is expressed by epithelial tumors of the gastrointestinal (GI) tract including the stomach, small and large intestines, colon, pancreas, and cervix. Ca19-9 mucin circulates in the blood and accumulates in ascites or pleural fluid of patients with GI cancer. Using an RIA kit specific for the Ca19-9 epitope, (Centocor/Fujirebio), 1 Unit of Ca19-9 corresponds to 0.59 ng/mL of the 116NS-19-9 antibody reactive determinants. This corresponds to ~1.7 U/ng of total protein or 1 KU equals 0.59 µg of Ca19-9 antigen. Other antibodies have been produced that recognize different determinants on the same molecule, including Ca50, Span-1, Ca242, Dupan-2, and SSEA-1, which are measured in terms of each specific antibodies reactive epitope units [1, 2].

### **Purity Ratio**

Bioprocessing controls the consistency of the Ca 19-9 purification by establishing limits on the purity ratio (U/ml/OD<sub>280nm</sub>). The purity ratio for the Ca 19-9 Ag purified from natural product and cell culture supernate material must be greater than  $1.0 \times 10^5$  U/m./OD<sub>nm</sub>. The purity of the natural sourced material can vary greatly due to patient variability but it must always have a purity ratio greater than 100,000 ( $1.0 \times 10^5$ ). The cell culture conditioned media is collected and chromatographed. Because we prepare the natural product and cell culture supernatant differently prior to purification, we also obtain differences in relative purity ratios.

### **ELISA**

Through the use of our antigens for several development projects involving testing GI Ag to develop GI MoAb HP (4250-8215 and 4250-8235) and testing against NS-1116 (Centocor), optimal coating concentrations are as follows:

<b>Antigen</b>	<b>Product Code</b>	<b>[U/mL]</b>
GI Ag HP Natural	4250-1000	1,000 U/mL
GI Ag HP CC	4250-2000	1,000 U/mL

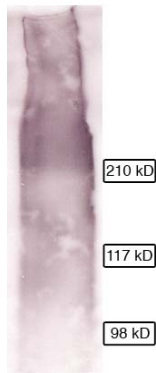
### Immunization

Based on our experience in generating monoclonal antibodies against the GI Ag produced at Bioprocessing, Inc., we have extensive experience with using the HP Ag for immunizations. For monoclonal antibodies, we normally immunize and boost with the following:

Antigen	Product Code	[U/mL]
GI Ag HP CC	4250-2000	30,000-50,000 U/mouse

### PAGE

We employ PAGE analysis to determine Ca19-9 purity for both natural product and cell culture supernatant. Loading 1-2 KU (0.5-1 µg) followed by staining with GelCode Blue (Thermo Fisher Scientific Inc., Rockford, IL). It is difficult to detect Ca19-9 based on the aggregates that it forms when in a concentrated solution. Western blot analysis of high pure Ca19-9 demonstrates the high degree of glycosylation present on the MUC-1 antigen (Figure 1).



**Figure 1. Western Blot of Pure Ca19-9 Antigen**

Purified Ca19-9 (500 U of catalogue #4250-2000) was subjected to western blotting with GI MoAb HP, Clone 4C11 (catalogue # 4250-8215), a monoclonal antibody that is specific for Ca19-9 epitope on MUC-1. Results demonstrate the purity in addition to the degree of glycosylation, as seen by the smearing pattern on the blot. 1

### Literature Cited

1. Ho, J.J., et al., *Expression of CA19-9, DU-PAN-2, and SPan-1 antigens on two types of normal salivary mucins*. *Oncol Res*, 1993. **5**(9): p. 347-56.
2. Lan, M.S., et al., *Co-expression of human cancer-associated epitopes on mucin molecules*. *Int J Cancer*, 1987. **39**(1): p. 68-72.
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4. Ho, J.J. and Y.S. Kim, *Serological pancreatic tumor markers and the MUC1 apomucin*. *Pancreas*, 1994. **9**(6): p. 674-91.

