



**Human Immunoglobulin M ELISA kit
Catalog # F170
Validation Summary
Report dated Jan. 16, 2002**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure Human Immunoglobulin M (hIgM). This data is intended to supplement and not replace user generated validation data. The data is representative of what a laboratory can expect to achieve when following the kit insert recommended protocols. Significant differences in these performance parameters may be indicative of problems with reagents, laboratory equipment, or technique and should be investigated before reporting results.

It is recommended that a user validation study include at least the following experiments to validate this kit for use with their product: (1) Each user should perform intra and inter assay precision experiments to establish their procedural proficiency. (2) Each user should perform recovery experiments using their test sample matrices and diluents. Such a study can be performed by adding known amounts of the 50ng/mL standard provided with this kit to the final product or diluent which are to be evaluated. Ideally, these test sample matrices should be devoid of any hIgM or have very low levels (< 0.5ng/mL) determined prior to adding the 50ng/mL standard. Such an experiment will establish the degree of sample or diluent matrix interference in the recovery of hIgM. (3) Laboratories should also perform dilutional recovery experiments on their actual samples. This experiment assumes that at least some of the test samples will have significant levels of hIgM. Such samples will be serially diluted by some appropriate diluent previously shown to give acceptable recovery. When diluted, samples should give essentially the same value at each dilution when multiplied by the appropriate dilution factor. This experiment establishes the condition of antibody excess for accurate quantitation and determines that typical process samples do not have hIgM in the “Hook Region” of the concentration response curve.

Methods: The protocol as defined in the kit insert was used in this validation.

Data References: Raw data for these experiments are recorded in Notebooks #1-hIgM pages 1-23.

Precision: Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the standard deviation by the mean value for a number of replicate determinations of two different control samples in the low and high concentration range of the assay. The design goal specifications are given in the last column of each experiment. While actual precision may vary from laboratory to laboratory and technician to technician it is recommended that all operators achieve precision below these design goals before reporting results.

Intra-assay				Inter-assay			
# of tests	Mean ng/mL	%CV	Design Goal Specification	# of assays	Mean ng/mL	%CV	Design Goal Specification
20	1.28	3.6	<10%	5	1.4	6.5	<12%
20	4.46	2.6	<8%	5	4.8	3.8	<10%
20	15.05	4.2	<8%	5	15.5	9.2	<10%

Sensitivity: The hIgM concentration corresponding to a signal 2 standard deviations above the mean of the zero standard is defined as the limit of detection (LOD). This was determined from 10 replicates of the zero standard. The mean signal of the zero standard plus 2 SD yielded a LOD of <177 pg/mL. The limit of quantitation (LOQ) is defined as the lowest concentration for which the CV is <20%. This is determined by performing a precision profile for the assay at several low concentration points and then interpolating that concentration which corresponds to a 20% CV. The LOQ was 250pg/mL.

Specificity: The antibodies used in this kit are essentially human μ chain specific and have been affinity purified against hIgM. Because it is difficult to obtain hIgM for either immunogen, antigen affinity purification, or assay calibrator purposes that does not have trace IgG or IgA contamination or IgG and IgA that does not have trace contamination with IgM, it is difficult to accurately determine cross reactivity. With these limitations considered, it is believed that cross reactivity for human IgG and human IgA is negligible (<0.1%) for most assay applications. It is recommended that each user consider testing their particular sample types and sample matrices for cross reactivity, or other types of interferences. Sera from various other animals were diluted 1:1000 and tested for apparent reactivity in this assay. As indicated in the table below, certain sera showed very minimal positive reactivity on a weight to weight basis, however at these very low levels it cannot be conclusively determined if this is true immunological cross reactivity or some non-specific increase in assay signal.

Animal Species	% Cross Reactivity (apparent hIgM/total protein)
Cat	Not detectable
Chicken	Not detectable
Cow	Not detectable
Dog	~0.009%
Goat	Not detectable
Hamster	~0.0004%
Horse	~0.0008%
Mouse	~0.0005%
Pig	~0.0008%
Rabbit	~0.0009%
Rat	~0.0008%
Sheep	Not detectable

Recovery/Interference Studies: Various buffer matrices have been evaluated by adding known amounts of the hIgM preparation used to make the standards in this kit. Because this assay is designed to minimize matrix interference most of these buffers yielded acceptable recovery defined as between 80-120%. In general, extremes in pH (<5.0 and >8.5) as well as some detergents like SDS and Tween can cause under-recovery. Very high concentrations of certain proteins can also interfere in accurate detection of hIgM. Each user should validate that their sample matrices yield accurate recovery by performing a similar experiment. For example, this experiment can be performed by diluting one part of the 50ng/mL standard provided with this kit into 4 parts of the sample matrix in question. Recovery should be on the order of 8 to 12 ng/mL hIgM.

Hook Capacity: Very high concentrations hIgM were evaluated for the hook effect. At concentrations exceeding 50ng/mL the apparent concentration of hIgM may read less than the 50ng/mL standard. The hook capacity, defined as that concentration which will give an absorbance reading less than the 50ng/mL standard was ~1mg/mL.