



**Mouse IgM ELISA kit  
Catalog # F090  
Validation Summary  
Report dated 6/8/01**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure Mouse IgM. 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. Such a study can be performed by adding known amounts of the 100ng/mL standard provided with this kit to the final product or any intermediate samples which are to be tested. Ideally these test sample matrices should be devoid of any Mouse IgM or have very low levels (< 0.5ng/mL) determined prior to adding the 100ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of Mouse IgM (3) Laboratories should also perform dilutional recovery experiments on their actual samples. This experiment assumes that at least some of the test samples from the purification process will have significant levels of Mouse IgM. 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 Mouse IgM in the “Hook Region” of the concentration response curve.

**Materials:** Kit Lot 26071E

**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-Mouse IgM pages 22-27.

**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	4.1	6.6	<10%	5	4.0	8.1	<12%
20	35.2	5.3	<8%	5	35.3	6.5	<10%

**Recovery/Matrix Interference:** The same Mouse IgM preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. Mouse IgM was added at 20ng/mL and tested in duplicate. In all cases, the zero for each sample buffer was within the limit of detection for the assay and thus the buffers themselves were considered to contribute 0 ng/mL of Mouse IgM. Acceptable recovery is specified as plus or minus 20% of the added Mouse IgM value. These data serve as examples of certain buffers or buffer components which may or may not give matrix interference. As shown below matrix interference can be either positive (false increase in Mouse IgM), or negative (false decrease in Mouse IgM). Each user is encouraged to test their sample matrices for recovery in a similar experiment. Adsorption of low concentrations of Mouse IgM to the vials or tubes used in making the spikes can cause under recovery. It is advisable to have some type of non-reacting carrier protein in the buffer to avoid adsorption losses. Your product protein or BSA can be used as blockers for Mouse IgM adsorption. If routine dilution of samples is required, it is recommended to use the same diluent as is used for the kit standards. This diluent can be purchased from *Cygnus Technologies* as product number I028.

Sample Buffer Matrix	Mouse IgM added ng/mL	Mouse IgM Recovered ng/mL	% Recovery (assayed/added x100)
0.05M TBS with 1% BSA, pH 7.2	20	20.4	102
0.05M PBS with 1% BSA, pH 7.4	20	21.2	106
Citrate phosphate with 1 mg/mL BSA, pH 7.0	20	19.2	96
0.05 M Carbonate, pH 9.5	20	16.8	84
Tris Glycine with 1% SDS	20	11.6	58
0.1M Acetate, 1% Triton, pH 4.5	20	18.2	92

**Sensitivity:** The Mouse IgM 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 250pg/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 350pg/mL.

**Specificity:** In sandwich ELISA, cross reactivity can manifest itself either as a false increase in Mouse IgM (positive cross reactivity) or as a false decrease in Mouse IgM (negative cross reactivity) when Mouse IgM present in the sample competes with the cross reactant for the kit antibodies. The following materials were tested for cross reactivity at the concentrations indicated both in the absence of Mouse IgM and in the presence of 5ng/mL Mouse IgM. None of these materials were found to yield any statistically significant false increase or decrease in apparent Mouse IgM concentrations. While no cross reactivity was detected in any of the substances tested it is recommended that each user test known materials in their sample matrices for cross reactivity in a similar experiment.

### Materials Not Cross Reactive for Mouse IgM

Substance	Concentration Tested
Mouse IgG1	2 mg/mL
Mouse IgG2a	“
Mouse IgG2b	“
Rabbit serum	1%
Bovine serum	“
Goat serum	“
Human serum	“



***Cygnus Technologies, Inc.***

4705 Southport Supply Road SE, Suite 208

Southport, NC 28461 USA

Tel: 910-454-9442 FAX: 910-454-9443

Email: [cygnustec@aol.com](mailto:cygnustec@aol.com)

Web: [www.cygnustechnologies.com](http://www.cygnustechnologies.com)

**Hook Capacity:** Very high concentrations Mouse IgM were evaluated for the hook effect. At concentrations exceeding 10,000ng/mL, the apparent concentration of Mouse IgM may read less than the 100ng/mL standard. Samples yielding signals above the 100ng/mL standard or suspected of having concentrations in excess of 10,000 ng/mL should be assayed diluted.