



**Cygnus Technologies, Inc.**

4705 Southport Supply Road, 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)

### **3 PROTEIN A KITS – WHICH TO USE**

*Cygnus Technologies* now offers 3 ELISA kits for detection of Protein A. Our first two kits, Cat # F050 and F050H have been sold for several years and have proven to be valid for use in detecting both natural and structurally conserved recombinant Protein A. The newest kit Cat # F400 was just released in September of 2007. Cat #F400, will better detect unnatural recombinant constructs of Protein A such as GE Healthcare's MabSelect SuRe™ ligand. Prior to the availability of this unique construct, all Protein A used for antibody purification was either natural Protein A derived from *Staphylococcus aureus* or recombinant Protein A (*E.coli* expressed) as patented and sold by Repligen. Repligen recombinant Protein A is very similar to natural Protein A with over 99% homology. Both Protein A preparations contain essentially the same 5 subunit binding domains (A, B, C, D, & E) and both have molecular weights of ~ 44.7kDa.

The new GE Healthcare MabSelect SuRe™ construct is a structurally unique molecule. MabSelect SuRe™ is derived only from the B binding domain of natural Protein A. It is a multimerized, tetramer of the B domain with a C-terminal cysteine introduced for site-directed immobilization. The molecular weight of MabSelect SuRe™ is 26.7kDa.

The *Cygnus* Protein A Kits, Cat #s F050 and F050H use antibodies made to natural Protein A with validation performed against only the natural and the highly conserved recombinant Protein A. With the introduction of MabSelect SuRe™ we have recently had the opportunity to evaluate its reactivity in these kits. Our preliminary data has shown that it has only about a 20% weight to weight reactivity relative to the Protein A standards in these kits. This 20% reactivity may be greater or less depending upon other factors such as the product antibody being purified, which may contribute to further interference or non-specificity.

The Cat. #F400 assay addresses the low reactivity to MabSelect SuRe™ seen in our other kits while incorporating several other improvements in the methodology. This new assay has replaced the biotinylated antibody used in our original kits with a different antibody directly labeled with HRP. Improvements include more sensitivity, simplified assay protocol, more standards, and less probability of product matrix interference. The #F400 kit detects natural, conserved recombinant, and MabSelect SuRe™ Protein A ligands essentially 1:1. Like the F050H assay the new F400 assay uses a sample denaturation step to overcome sample/product antibody interferences. Because so many laboratories have successfully validated and are routinely using our earlier kits #F050 & #F050H, we will continue to offer these kits indefinitely. First time evaluators of Protein A detection methods are encouraged to evaluate the new #F400 kit regardless of which type of Protein A they use.