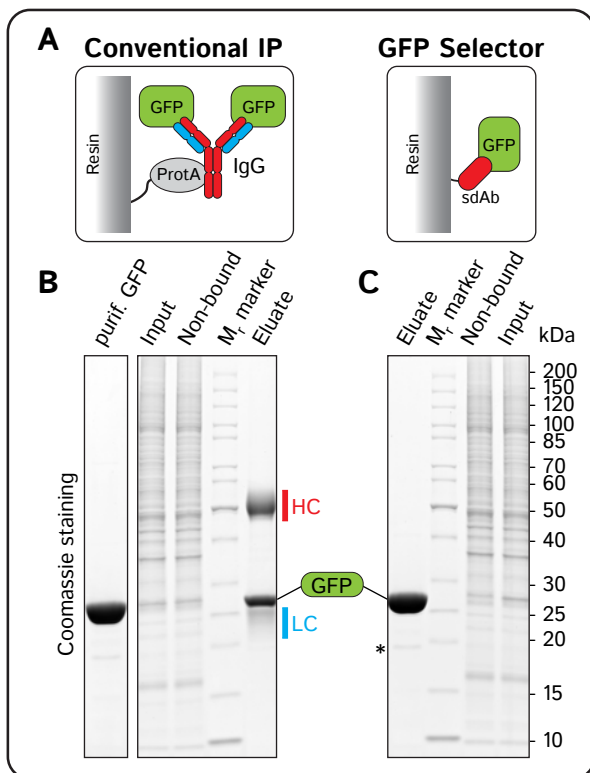


# GFP Selector

## The next generation of GFP pull-down resins

Application Note 09/17

NanoTag Biotechnologies recently launched its novel GFP Selector resin for easy and efficient pull-down of GFP fusion proteins. Here, we compare GFP Selector to conventional IgG-based immunoprecipitation techniques and also to a competitor's product (CT). We show that GFP Selector features not only superior capacity for GFP-tagged proteins, but also a strikingly lower non-specific background binding. GFP Selector thus offers clear benefits in comparison to both, conventional IgG-based IPs and to the competitor CT's product.



**Figure 1: Comparison of conventional and GFP Selector-based immunoprecipitation**

**A.** Schematic comparison of a conventional immunoprecipitation using a GFP-specific antibody coupled to Protein A (ProtA, left) and a GFP Selector-based IP (right).

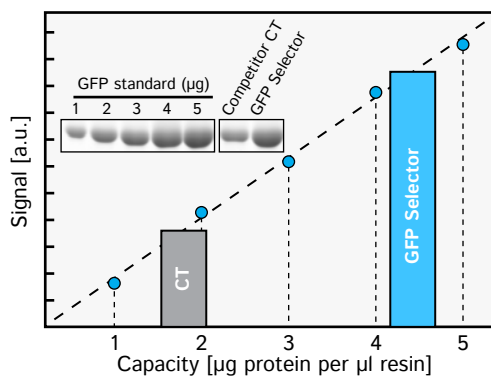
**B.** Conventional immunoprecipitation of GFP from HeLa lysate using antibodies coupled to a Protein A resin. During elution with SDS sample buffer, IgG light and heavy chains (LC/HC) are eluted from the resin together with the antigen.

**C.** Due to the covalent and stable linkage of an GFP-specific sdAb to the agarose support, bound GFP fusion proteins are eluted from GFP Selector without contaminating IgG chains.

\* Band originating from maturation of GFP.

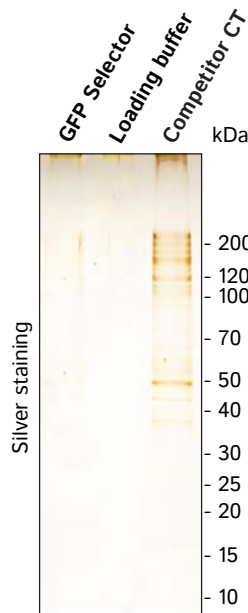
**Immunoprecipitation of GFP fusion proteins** is typically performed using GFP-specific antibodies that are non-covalently immobilized on a solid support via Protein A or Protein G (**Fig. 1A**, left). During elution with SDS sample buffer, the antibody is denatured and detaches from the resin together with the GFP fusion protein and its interaction partners. The eluate therefore typically contains significant amounts of IgG heavy and light chains (**Fig. 1B**). These contaminations often hamper a clear identification of specific interaction partners and may cause drastic problems in downstream applications like Western blot or mass spectrometry.

**NanoTag Biotechnologies** now introduced **GFP Selector**, an innovative high quality resin for efficient and reproducible pull-down of GFP fusion proteins. GFP Selector is based on a **single-domain antibody (sdAb) developed in-house**, that recognizes GFP and most GFP derivatives with **high specificity** and **picomolar affinity**. Importantly, GFP fusion proteins can be eluted from GFP Selector without contaminating IgG chains (**Fig. 1C**). Proteins specifically interacting with the GFP fusion protein can thus easily be identified. Due to the monoclonal nature of the sdAb and its novel, oriented and stable attachment on the agarose-based solid support, GFP pull-downs using GFP Selector are efficient and highly reproducible. In contrast, GFP antibodies used for conventional IPs are often polyclonal, i.e. the quality and specificity of the antibody may vary on a batch-to-batch basis.



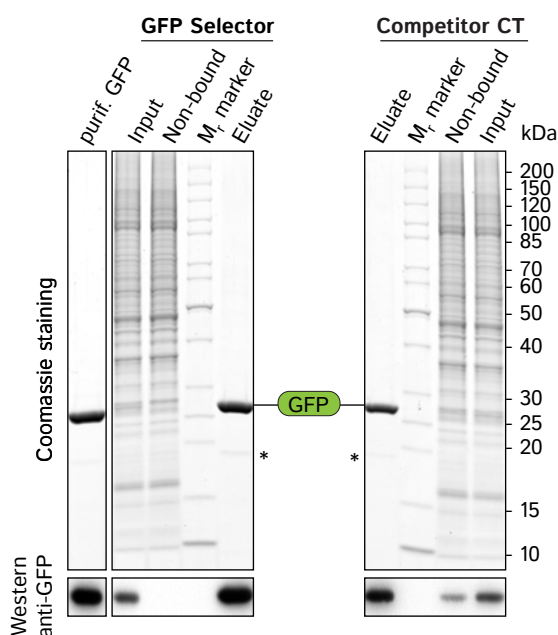
**Figure 2: GFP Selector features superior capacity.**

GFP Selector and the competitor's product were incubated with saturating amounts of recombinant GFP. Material bound to 1  $\mu$ l of resin was analyzed by SDS-PAGE and Coomassie staining. For quantification, signal intensities were compared to a standard curve obtained with defined amounts of GFP.



**Figure 3: Unspecific binding of proteins from a mock lysate**

Same amounts of GFP Selector and the competitor's product were incubated with HeLa lysate devoid of any GFP-tagged protein. After washing, aliquots corresponding to 2  $\mu$ l of resin were resolved by SDS-PAGE and analyzed by silver staining. Note that GFP Selector clearly shows lower background binding as compared to the competitor's product.



**Figure 4: Pull-down from HeLa lysate using GFP Selector and a competitor's product.**

Same amounts of GFP Selector or the competitor's product were incubated with 1 ml of a GFP-containing HeLa lysate. Aliquots of input and non-bound material as well as corresponding eluate fractions were analyzed by Coomassie staining and Western blot. Bands correspond to 1  $\mu$ l of input or non-bound fractions and 0.5  $\mu$ l (Coomassie) or 0.05  $\mu$ l (Western blot) of resin. Note that GFP was undetectable in the non-bound fraction only if GFP Selector was used.

\* Band originating from maturation of GFP.

The yield and efficiency of conventional pull-down systems is often limited by the availability of the primary antibody and the IgG-binding capacity of the IgG-binding solid phase. GFP Selector features a **high capacity for GFP fusion proteins** (> 4  $\mu$ g GFP per  $\mu$ l of resin, **Fig. 2**). It thereby clearly outcompetes both conventional IgG-based methods (see **Fig. 1**) and competitor CT's product (**Fig. 2**).

NanoTag Biotechnologies uses a unique chemistry on a high-quality **agarose-based solid support**. This allows for a **covalent and oriented attachment** of high-affinity sdAbs and minimizes non-specific binding of proteins from complex lysates. As a result, GFP Selector allows the user to obtain significantly cleaner pull-downs of GFP-tagged proteins (**Fig. 3**). The high capacity and **low non-specific binding** lead to a significantly **improved signal-to-noise ratio**, which facilitates the straight-forward identification of proteins specifically binding the GFP-tagged bait.

In a side-by-side comparison, only GFP Selector was able to bind all target protein from a HeLa lysate containing GFP (**Fig. 4**). The **high affinity** and **superior capacity** of GFP Selector thus efficiently prevents unwanted loss of GFP-tagged target proteins.

**Summary:** GFP Selector outperforms both, competitor CT's product and conventional immunoprecipitation with respect to binding capacity and signal-to-noise ratio. It is thus the first choice for pull-down of GFP fusion proteins from complex lysates.