iba Solutions For Life Sciences

Protein Production & Assays

Application Note

GFP SELECTOR

The next generation of GFP immunoprecipitation resins

Introduction

Immunoprecipitation (IP) 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 GmbH now introduced GFP Selector, an innovative high quality resin for efficient and reproducible IP 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.

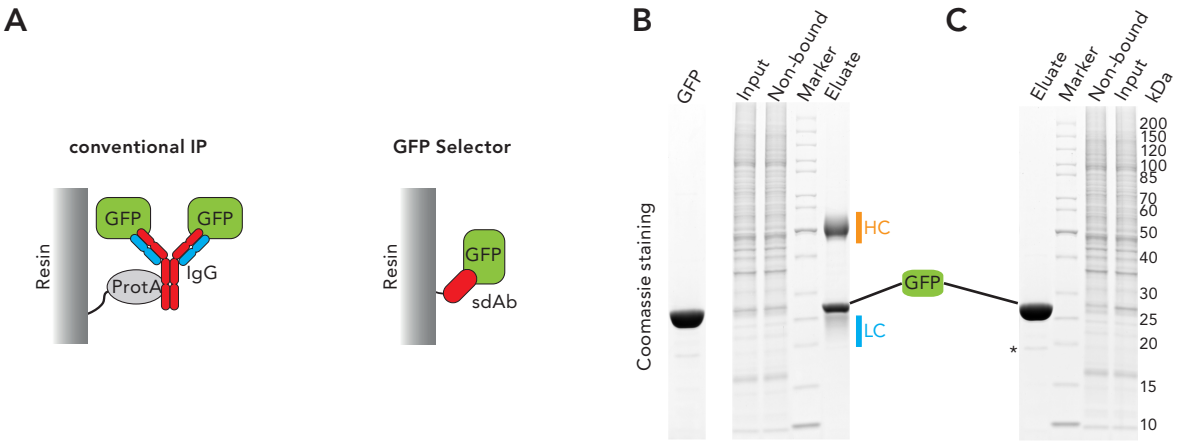

Figure 1. Comparison of conventional and GFP Selector-based immunoprecipitation (IP)
(**A**) Schematic comparison of a conventional IP using a GFP-specific antibody coupled to Protein A.
(**B**) Conventional IP of GFP from HeLa lysate using antibodies coupled to a Protein A resin. During elution with 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 Resin without contaminating IgG chains. * Band originating from maturation of GFP.

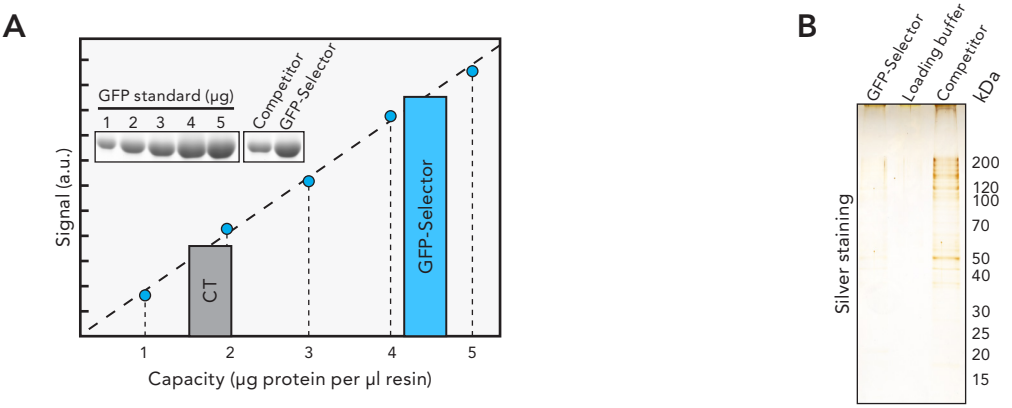

Figure 2. GFP Selector features superior capacity combined with high specificity

(**A**) GFP Selector and the competitor's product were incubated with saturating amounts of recombinant GFP. Material bound to 1 µl 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.

(**B**) 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 µl 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.

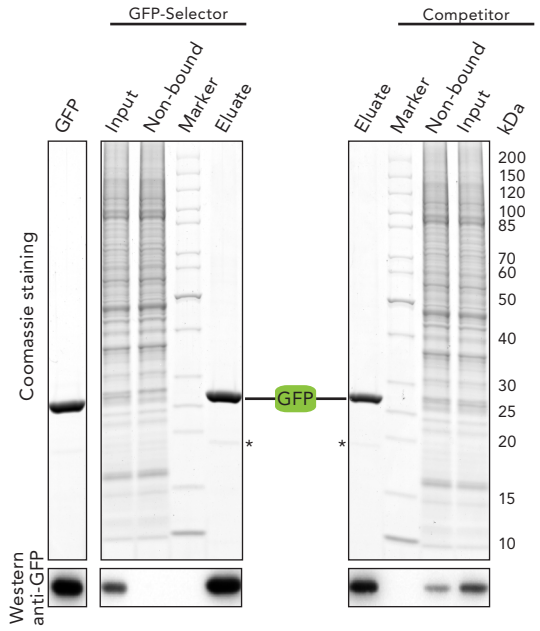

Figure 3. Immunoprecipitation from HeLa lysate using GFP Selector and a competitor's product

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 µl of input or non-bound fractions and 0.5 µl (Coomassie) or 0.05 µl (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.

Results and discussion

The yield and efficiency of conventional IP 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 µg GFP per µl of resin, Fig. 2A). It thereby clearly outcompetes both the competitor's product (Fig. 2) and the conventional IgG-based method (see Fig. 1).

NanoTag Biotechnologies GmbH 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 IP of GFP-tagged proteins (Fig. 2B). 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. 3). The high affinity and superior capacity of GFP Selector thus efficiently prevents unwanted loss of GFP-tagged target proteins.

Summary

GFP Selector outperforms both, the competitor'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.

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