# Manual of GFP IP Kit

## Cat. No. GFP-IP

| Catalog No. | **GFP-IP-50** | **GFP-IP-200** |
|---|---|---|
| Size | **50 rxns** | **200 rxns** |
| Price (USD) | $250 | $920 |
| Biotinylated control IgY (0.2mg/ml) | 250ul/$25.00 order separately | 4x250ul/$90.00 order separately |

**Introduction**

This kit is for capturing GFP and its fusion proteins from cell lysate. The combination of biotinylated chicken IgY against GFP protein as IP antibody and streptavidin conjugated magnetic beads as isolating matrix confers the kit with several advantages:

1) The chicken IgY anti GFP antibody in this kit is antigen affinity purified, which will greatly reduce the use of the beads, meanwhile minimize the non-specificity, as compare to using total IgG or total IgY in IP.

2) Because of the extremely high affinity of streptavidin to biotin, the streptavidin-magnetic beads can capture biotinylated IgY GFP/GFP complex at a very high efficiency, which will increase the sensitivity of capture. This kit will allow users to capture as low as 1ng GFP protein and as high as 330ng GFP protein.

3) The Fc region of chicken IgY is very different from Fc region of mammalian IgG. When protein detecting antibodies from mammalian species are used in Western Blot after the immunoprecipitation, it will not show undesired bands since anti-IgG secondary antibodies do not bind or bind very little to IgY.

**Kit contents**

| Catalog No. | **GFP-IP-50** | **GFP-IP-200** |
|---|---|---|
| Size | **50 rxns** | **200 rxns** |
| Biotinylated IgY anti-GFP (0.2mg/ml) | 260ul | 1020ul |
| Streptavidin-Magbeads (10mg/ml) | 65ul | 250ul |
| Eluting solution | 1.25ml | 5ml |
| Neutralization solution | 0.2ml | 0.8ml |

**Reminding**

1. Users must use proper ways to prepare cell lysate to assure 1) chromosomal DNA is disrupted so that the lysate is not glutinous, and 2) proteolysis in the lysate is inhibited.

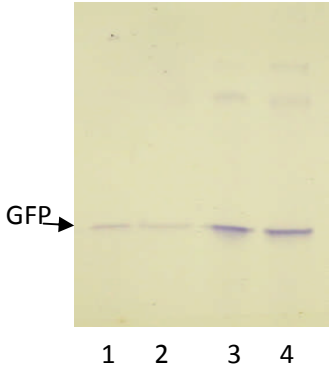
2. This capture protocol is developed using round bottom 96-well plate, users may choose other formats, such as microcentrifuge tubes or PCR tubes.
3. For short period storage (up to 2 weeks), store the whole kit at 4°C; for long term storage, take out the tube labeled **Biotinylated IgY anti GFP** and store at -20°C, and store the rest at 4°C. Avoid frequent frozen-thaw cycles.

**Protocol**

1. Pretreat Streptavidin Magbeads

   Fully suspend beads by vortex and take desired volume of the beads suspension (12ug for each capturing reaction). Remove the liquid on magnetic separator (provided by user), and wash the beads three times with PBST (0.05% Tween 20), 10 volumes of beads suspension each time, using magnetic separator. Resuspend the beads in PBST to make **2mg/ml**. It is ready to use.
2. To **100-200ul** clear cell lysate, add **5ul (0.2ug/ul)** Biotinylated IgY anti-GFP, mix well.
3. Incubate at room temperature for **1 hour** with shaking.
4. Add **6 ul (2ug/ul)** preteated streptavidin Magbeads (**fully suspended**) to the mixture, mix well.
5. Incubate at room temperature for **30 minutes** or **4°C for overnight** with shaking. During the incubation, pipette the mixture couple times in every **10 minutes. (if tube is used, the incubation can be performed on end-to-end rotator or other appropriate equipment, then no need for interval pipetting)**
6. Isolate the beads from solution on magnetic separator. (you may save the liquid)
7. Wash the beads with **200ul** PBST (0.05% Tween 20) three times, by using magnetic separator.
8. After wash, add **10 or 20ul** Eluting solution, pipette to completely suspend the beads. Then separate the solution from the beads on magnetic separator. Transfer the elutant to a new tube that has been pre-added with **1 or 2ul** Neutralization solution. (with this eluting condition, no or very little IP antibody will be eluted, which greatly reduces interference from the IP antibody bands in Western Blot where the 1st antibody used is from the same species as the IP antibody.) The elutant is now ready for downstream analysis.

**Example**




**Western Blot analysis of the capture efficiency**

1: 1ng GFP input
2: 1ng GFP IP captured in 100ul tissue lysate
3: 10ng GFP input
4: 10ng GFP IP captured in 100ul tissue lysate
1st antibody: IgY anti-GFP