

### Product information

**Product number:** AS05 070  
**Clonality:** Polyclonal  
**Raised in:** Rabbit  
**Storage instructions:** -20°C.  
Aliquoting is recommended.

Short term storage in + 4°C.

*Please, avoid freezing and thawing of antibodies. Make aliquots instead.*

*Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.*

### Background

**Rubisco** - universal and absolutely essential carbon fixation enzyme that is conserved across oxygenic photoautotrophs and other groups. The RbcL (Rubisco large subunit) protein is hyperabundant and regulation of RbcL content under different conditions or stress responses sets a limit on the maximum capacity for CO<sub>2</sub> uptake in a population or community.

Proteins involved in conversion of solar energy into chemical bonds:

**PsaC** – protein of multisubunit protein complex - Photosystem I, which harnesses light energy required for photosynthesis to occur.

**PsbA** – protein of multisubunit complex - Photosystem II - the ultimate source of almost all biosynthetic reductant in the biosphere. PsbA (D1) protein is rapidly cycled under illumination in all oxygenic photobionts. Tracking PsbA pools using the Global PsbA Antibody shows the functional content of Photosystem II in a wide range of samples.

**Lhcb1** - harnesses light energy for photosynthesis (Photosystem II)

**AtpB** – highly conserved across the beta subunits of known F-type ATP Synthases from chloroplasts, mitochondria and most bacteria. The ATP Synthase complex is essential for the synthesis of ATP from ADP and free phosphate.

### Product information - Primary antibodies:

| Product number: | Product name:              | Quantity:  | Recommended Dilution:     | Related product:        |
|-----------------|----------------------------|--|---------------------------|-------------------------|
| AS01 004        | Anti-Lhcb1 antibody*       | Lyophilized. Please add 10 µl of sterile water to reconstitute antibodies. | 1:5 000 for ECL detection | No standard available   |
| AS03 037        | Anti-RbcL global antibody* |  |                           | AS01 017S RbcL standard |
| AS10 939-10     | Anti-PsaC global antibody* |  |                           | AS04 042S PsaC standard |
| AS05 084        | Anti-PsbA antibody*        |  |                           | AS01 016S PsbA standard |
| AS05 085        | Anti-AtpB antibody*        |  |                           | AS03 030S AtpB standard |

\* Primary antibodies raised in rabbit

**Recommended secondary antibody:** Anti-rabbit (HRP)

### Product information - Protein standards/positive controls:

| Product number: | Product name:                   | No of vials: | Reconstitution: | Amount to load on the gel: | Size:     |
|-----------------|---------------------------------|--------------|-----------------|----------------------------|-----------|
| AS01 016S       | PsbA protein standard           | 1            | Add 90ul        | 10 µl (0.25 pmol/ µl)      | 41.5 kDa* |
| AS01 017S       | RbcL (Rubisco) protein standard | 1            | Add 90ul        | 5 µl (0.056 pmol/ µl)      | 52.7 kDa  |
| AS03 030S       | AtpB protein standard           | 1            | Add 90 ul       | 10 µl (0.27 pmol/µl)       | 53.1 kDa* |
| AS04 042S       | PsaC protein standard           | 1            | Add 90ul        | 10 µl (0.09 pmol/µl)       | 11.5 kDa* |

\*Larger than native protein due to the addition of His-tag.

**Quantity and format:** Lyophilized. Please add sterile water for reconstitution of antibodies and protein standards

#### Product Support:

<http://www.agrisera.se/protocols/protocols.shtml>

**E-mail:** [joanna@agrisera.se](mailto:joanna@agrisera.se)

## General Information

### **Suggested Treatments:**

#### **Low light vs. High light**

Shift from low or moderate light (50-100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) to high light (500-2000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , or nearly full sunlight).

**Note:** Depending on the species and the developmental stage this will lead to large changes in protein profiles, usually within a few hours and is reflected in detectable differences in LHC & RbcL content, and also differences in the PsbA/PsaC ratio (PSII:PSI).

-PsbA breakdown products will become more prominent, and the pool of mature PsbA usually declines

-there may be declines in antenna proteins, and sometimes increases in RbcL, if the stress is not too harsh.

For higher crop plants, it is probably the most generally reliable, rapid way to provoke detectable changes in protein content, since the PsbA/D1 pool will usually decline.

#### **Changes in nutritional status**

Shifting from nutrient replete to low-nutrient conditions will cause gradual declines in LHC & RbcL content.

#### **Etiolation vs. greening**

Characteristic change, usually PSI (PsaC) accumulates before PSII (PsbA) and LHCII can be observed.

#### **Developmental stages**

Newly emerged, young & old leaves will show different profiles, as will (of course) leaves & roots.

#### **Suggested material:**

Plants (spinach, sunflower, pea), Algae or cyanobacteria.

### **Material preparation and extraction:**

Tissue samples of the size required for later extraction can be frozen in foil sachets and stored at  $-80^{\circ}\text{C}$ .

Extraction buffer:

- LiDS is recommended over SDS for extraction involving cold steps.
- Protease inhibitor: PEFABLOC. For a teaching lab, this may not be strictly necessary, however

if extracts are to be refrozen and reused, degradation becomes an issue and addition of protease inhibitor is advised.

Recommended extraction volume: 150 mg of leaf tissue into 500  $\mu\text{l}$  of extraction buffer (see appendix). After the extraction by grinding and/or sonication the mixture is centrifuged (full speed in microfuge for 2 min) to separate solubilized extract from bulk leaf material. The recovery of extract should be about 300  $\mu\text{l}$ , depending on the type of leaves. This gives an extract at a concentration of roughly 100  $\mu\text{g chl/ml}$  (0.1  $\mu\text{g chl}/\mu\text{l}$ ).

Load per well: ca. 0.5  $\mu\text{g chl}$  (so ca. 5  $\mu\text{l}$  of the extract).

So 150 mg of leaf tissue should give a recovered extract of 300  $\mu\text{l}$  @ 0.1  $\mu\text{g chl}/\mu\text{l}$  = 30  $\mu\text{g chl}$ , sufficient for about 60 lane loads of 0.5  $\mu\text{g chl}/\text{lane}$  for immunoblotting

The loads can be reduced easily down to 0.2  $\mu\text{g chl}$  (for mini gels) or even lower (down to 0.01  $\mu\text{g chl}$  in some cases using ultrasensitive detection methods). Lighter loads are recommended for some abundant proteins detected with strong antibodies (like anti-RbcL).

Alternatively, for ease of extraction it can be scaled up to 1 g of tissue in 4 ml of extraction buffer. Loads can be prepared based on: chlorophyll, protein or leaf area. Chlorophyll is suitable and simple loading parameter

for determination of the level of the photosynthetic apparatus, which accounts for most leaf protein. Therefore loading by equal chlorophyll can show both qualitative and quantitative changes in a protein profile. For leaves, loads by equal area are recommended, as a leaf is nearly 2-dimensional and photosynthetic capacity per leaf area is a useful ecophysiological parameter.

### **Spectrophotometric chl determination**

Extract chl from ca. 100  $\mu$ l of aqueous extract into 900  $\mu$ l of 80% acetone saturated with  $MgCO_3$ .

Measure at  $A_{663}$  and  $A_{750}$  (Blank=1 ml 80 % acetone).

$$[Chl a](\mu g \cdot ml^{-1}) = 12.7 (A_{663} - A_{750}) \times 1000/x$$

### **Quantitation**

It is recommended to run 3 lanes of quantitated standard to generate a standard curve. It can be helpful to perform trial runs to get the quantitation standards and samples in the same range.

Immunoblotting with primary and secondary antibodies and ECL shows linearity of detection over about 1 order of magnitude. Bands are detected over a much wider range, but the pseudo-linearity load/response region is only about 10 fold.

### **Western Blot development protocol**

Please, check on AgriSera homepage under the following link:

<http://www.agrisera.com/protocols/westernglobal.shtml>

### **Appendix**

#### **Extraction / Sample Buffer Formulation:**

4X stock:  
0.56 M Tris Base  
0.42 M Tris Hcl  
8% LDS  
40% glycerol (w/v)  
2.0 mM EDTA (start with an EDTA stock (eg. 0.5M, prepared in advance in order to solubilize).

The final (1X) composition is therefore:

140 mM Tris base,  
105 mM Tris-HCl,  
0.5 mM EDTA,  
2% LDS,  
10% glycerol.