product **AS03 037**  
RbcL | Rubisco large subunit, form I and form II

**product information**

<table>
<thead>
<tr>
<th>Background</th>
<th>This antibody is especially suitable for quantifying of Rubisco in plant and algal samples. Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the rate-limiting step of CO2 fixation in photosynthetic organisms. It is demonstrably homologous from purple bacteria to flowering plants and consists of two protein subunits, each present in 8 copies. In plants and green algae, the large subunit (~55 kDa) is coded by the chloroplast rbcL gene, and the small subunit (15 kDa) is coded by a family of nuclear rbcS genes.</th>
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</thead>
<tbody>
<tr>
<td>Immunogen</td>
<td>KLH-conjugated synthetic peptide conserved across all known plant, algal and (cyano)bacterial RbcL protein sequences (form I L8S8 and form II L2), including Arabidopsis thaliana AtCg00490, Hordeum vulgare P05698, Oryza sativa P0C510, Chlamydomonas reinhardtii P00877, Synechococcus PCC 7920 A5CKC5</td>
</tr>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Purity</td>
<td>Serum</td>
</tr>
<tr>
<td>Format</td>
<td>Lyophilized</td>
</tr>
<tr>
<td>Quantity</td>
<td>50 µl</td>
</tr>
<tr>
<td>Reconstitution</td>
<td>For reconstitution add 50 µl of sterile water.</td>
</tr>
<tr>
<td>Storage</td>
<td>Store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.</td>
</tr>
</tbody>
</table>

**Tested applications**  
Immunofluorescence/confocal microscopy (IF), Immunogold (IG), Tissue Printing (TP), Western Blot (WB)

**Related products**  
- AS03 037A | anti-RbcL | Rubisco large subunit, form I and form II (50 µg affinity purified)  
- AS03 037-HRP | anti-RbcL | Rubisco large subunit, form I and form II (40 µg, HRP-conjugated)  
- AS15 2995 | anti-RbcL II | Rubisco large subunit, form II (50 µl), rabbit antibody  
- AS15 2995S | RbcL II | Rubisco form II positive control/quantitation standard  
- AS01 017 | anti-RbcL | Rubisco large subunit, form I, chicken antibody  
- AS01 017S | Rubisco protein standard for quantitative western blot or positive control  
- AS03 037PRE | Rubisco large subunit, pre-immune serum  
- AS09 400 | Rubisco quantitation kit  
- AS15 2994 | Rubisco ELISA quantitation kit  
- AS07 218 | anti-Rbcs | 557 kDa hexadecamer, rabbit antibody to a whole protein  
- AS07 259 | anti-RbcS | Rubisco small subunit (SSU), rabbit antibody  
- AS07 222 | anti-RbcS | Rubisco small subunit (SSU) from pea, rabbit antibody  

**Additional information**  
anti-RbcL can be used as a cellular [compartment marker] of plastid stroma (cytoplasm in cyanobacteria) and detects RbcL protein from 31.25 fmole. As both forms (I and II) are detected it is suitable for work with samples from Dinoflagellates, Haptophytes and Ochrophytes (diatoms, Raphidophytes, brown algae) as well as higher plants. This antibody together with Agrisera Rubisco protein standard is very suitable to quantify Rubisco in plant and algal samples.

Example of a simultaneous western blot detection with RbCL, PsbA and PsaC antibodies.
This product can be sold containing ProClin if requested

**Application information**

**Recommended dilution**
- Immunofluorescence/confocal microscopy (IF), 1: 1000 (IG), 1: 250 for images see Prins et al. (2008), detailed protocol available on request, 1: 800 (TP), 1: 5000 - 10 000 (WB)

**Expected | apparent MW**
- 52.7 kDa (Arabidopsis thaliana), 52.5 kDa (cyanobacteria), 52.3 (Chlamydomonas reinhardtii)

**Confirmed reactivity**

**Predicted reactivity**
- di and monocots, conifers, mosses, liverworts, welwitschia, green algae, red algae, brown algae, cryptomonad, cyanobacteria including prochlorophytes, gamma-proeobacteria, beta-proteobacteria, alpha proteobacteria, Suadae glauca

**Not reactive in**
- no confirmed exceptions from predicted reactivity known in the moment

**Additional information**
- This antibody was used in:
  - Western blot and tissue printing during a student course Ma et al. (2009).

**Selected references**
Agitation. Membrane was cut in half and left part was incubated in anti-rabbit DyLight® 550 secondary antibody (AS11 1782) diluted to 1:2 000.

Antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 (rabbit anti-Rubisco AS03 037) for 1.5 h at RT with agitation. The Amersham WB PVDF using wet transfer. Blots were blocked with 2% Amersham ECL Blocking Agent for 1h at room temperature (RT) with agitation. Membrane was incubated in the primary antibody at a dilution of 1: 10 000 (rabbit anti-Rubisco AS03 037) for 1.5 h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Membrane was cut in half and left part was incubated in anti-rabbit DyLight® 550 secondary antibody (AS11 1782) diluted to 1:2 000.


Sevseg et al. (2013).Exploring the effect of salinity changes on the levels of Hsp60 in the tropical coral Seriatopora caliendrum. June 29. (Symbiodinium sp. antibody reactivity)


Li et al. (2012). MAP Kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in Arabidopsis. New Phytol. ahead of print - RbcL antibody used as loading control.

Zhao et al. (2011). Expansins are involved in cell growth mediated by abscisic acid and indole-3-acetic acid under drought stress in wheat. Plant Cell Rep. Nov (RbcL antibody used as a loading control)


application example

0.25 µg of chlorophyll a/ lane from Spinacia oleracea (1), Synechococcus PCC 7942 (2), Cyanophora paradoxa (3), Heterosigma akashiwo (4), Thalassiosira pseudonana (5), Euglena gracilis (6), Micromonas pusilla (7), Chlamydomonas reinhardtii (8), Porphyra sp (9), Gonyaulax polyedra (10), Emiliania huxleyi (11) extracted with PEB (AS09 300), were separated on 4-12% NuPage (Invitrogen) LDS-PAGE and blotted 1h to nitrocellulose. Filters were blocked 1h with 2% low fat milk powder. Antibody incubations were followed by washings in TBS-T. All steps were performed at RT with agitation. Blots were developed for 5 min with ECL Advance detection reagent according the manufacturers instructions (GE Healthcare). Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

1 µg of chlorophyll from Cryptophyte samples (1,2) and 1 µg of chlorophyll (3) or 10 µg of total protein (4) from Arabidopsis thaliana leaves extracted either with 2ml of 100 mM Tris-HCl, 50 mM EDTA, 250 mM NaCl, 0.05% SDS (Sample 1) or 10 mL of 50 mM Hepes-KOH (pH 7.8), 330 mM sorbitol, 10 mM EDTA, 5 mM NaCl, 5 mM MgCl2, 5 mM sodium ascorbate and 0.2% BSA (Sample 2). Samples were denatured with 1:1 Amersham WB Loading Bufferr at 70C for 10 min and were separated on pre-casted 13.5% Amersham WB gel and blotted for 30 min to Amersham WB PVDF using wet transfer. Blots were blocked with 2% Amersham ECL Blocking Agent for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 (rabbit anti-Rubisco AS03 037) for 1.5 h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Membrane was cut in half and left part was incubated in anti-rabbit DyLight® 550 secondary antibody (AS11 1782) diluted to 1:2 000.
in TBST for 1h at RT with agitation. The blot was scanned using Cy3 channel of Amersham WB System.

Courtesy Dr. Małgorzata Wessels, Agrisera

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**application example**

2 µg of total protein from various plant extracts (1-5) extracted with PEB (AS08 300) separated on 4-12% NuPage (Invitrogen) LDS-PAGE and blotted 1h to PVDF. Markers MagicMarks (Invitrogen) (M) and Rubisco protein standard (AS01 017S) at 0.0625 pmol, 0.125 pmol, 0.25 pmol.

Following standard western blot procedure this image has been obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad). The contour tool of the software is used to the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample.

**Note:** Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 ug total protein, depending on the abundance of the target protein.