

Product no **AS16 ECL-S** AgriseraECL SuperBright

Product information

AgriseraECL SuperBright for Western Blot detection is a high quality substrate for detection of horseradish peroxidase enzyme activity at **extreme low femtogram** levels, offering low background and superior signal to noise ratio.

It is a ready to use 2 component system with low background and superior signal to noise ratios and highest sensitivity.



This reagent offers extreme low femto level sensitivity combined with low background and superior signal to noise ratios.

Storage Store at 2 °C to 8 °C. Mixed working reagent is stable for several days at room temperature or at 4 °C. Exceptional lot to lot consistency.

Shelf life is 24 months when stored in the dark at 2 °C to 8 °C. Keep container tightly closed. Store away from heat or light.

Application information

Additional information | User Instruction

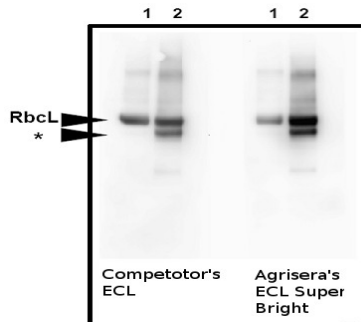
- Store reagents A and B in the darkness at 4-8 °C.
- Mix equal volumes of reagent A and B (chemiluminescent substrate) in a **clean container** and equilibrate to room temperature 30 minutes before use.
- Prepare your membrane prior addition of chemiluminescent substrate, by a wash with a buffer used in your protocol (PBS or TBS or TBST-T). This will allow to remove any background prior to substrate contact.
- Optimal visualization may be obtained up to 20 minutes after substrate contact. Usually, incubation for 2-5 is optimal.
- Remove excess substrate by filter paper.
- Cover blot with clear plastic wrap or sheet protector and expose either with x-ray film or CCD camera.

In some cases Tween can quench the reaction.
For best results clean containers and high quality water has to be used.

HS code for this product is: 3822.00.0002.

- Selected references**
- [Naranjo](#) et al. (2021) NTRC Effects on Non-Photochemical Quenching Depends on PGR5. *Antioxidants* (Basel). 2021 Jun 3;10(6):900. doi: 10.3390/antiox10060900. PMID: 34204867; PMCID: PMC8229092.
 - [Ferrero](#) et al. (2019). Class I TCP transcription factors target the gibberellin biosynthesis gene GA20ox1 and the growth promoting genes HB11 and PRE6 during thermomorphogenic growth in Arabidopsis. *Plant Cell Physiol.* 2019 Jul 11. pii: pcz137. doi: 10.1093/pcp/pcz137.

Application example



10 µg of total protein from *Arabidopsis thaliana* leaf (1), *Hordeum vulgare* leaf (2) were extracted with Protein Extraction Buffer PEB ([AS08 300](#)). Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and kept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to PVDF using tank transfer. Blots were blocked immediately following transfer in 5% non-fat milk dissolved in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in Agrisera anti-RbcL primary antibody ([AS03 037](#)) at a dilution of 1:20 000.

The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (goat anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody [AS09 602](#), Agrisera) diluted to 1:20 000 following by washes and detection using [Agrisera ECL SuperBright](#) detection reagent. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 60 seconds.

Note: In the barley sample, a well characterised 44 kDa degradation product is observed ([Kokobun et al. 2002](#)).

Caution:

Do not add any protein or HRP enzyme to reagent solution.

Optional: wash your tube, microtiter plate or membrane with 0.2 M solution of Sodium Phosphate, Dibasic, in Deionized Water. (Initial pH may be approximately 9). Bring the pH down to 8.4 with the slow addition of a 0.2M solution of Sodium Phosphate, Monobasic, in water.