Product no AS16 ECL-SN
Agrisera ECL kit (Bright/SuperBright) (100 ml)

Product information

Background
Agrisera ECL kit (Bright/SuperBright) is a Western Blot detection kit, that combines two chemiluminescent reagents with different detection range for visualization of horseradish peroxidase enzyme activity.

It is a ready to use 2 component system with low background and superior signal to noise ratios and moderate to highest sensitivity offered by:

- Agrisera ECL SuperBright
  Extreme low femtogram detection

- Agrisera ECL Bright
  Low pico to mid femtogram detection

Storage
- 18 month shelf life (Agrisera ECLSuperBright) at 2°C to 8°C.
- 24 month shelf life (Agrisera ECLBright) 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.

Related products
- Low pico to mid femtogram detection:
  AS16 ECL-N-10 | Agrisera ECL Bright (10 ml, trial pack)
  AS16 ECL-N-100 | Agrisera ECL Bright (100 ml)
  AS16 ECL-N-1L | Agrisera ECL Bright (1L)

- Extreme low femtogram detection:
  AS16 ECL-S-10 | Agrisera ECL SuperBright (10 ml, trial pack)
  AS16 ECL-S-100 | Agrisera ECL SuperBright (100 ml)
  AS16 ECL-S-1L | Agrisera ECL SuperBright (1L)

- Low pico to mid femtogram and extreme low femto detection in one set:
  AS16 ECL-SN-10 | Agrisera ECL kit (Bright/SuperBright), 10 ml trial pack
  AS16 ECL-SN-1L | Agrisera ECL kit (Bright/SuperBright), 1L

Additional information
- This set combines reagents with low pico to mid femtogram and extreme low femtogram detection range combined with low background and superior signal to noise ratios.

Application information

Additional information
- 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 is obtained up to 20 minutes after substrate contact. Incubation for 2 to 5 minutes is usually enough.
- 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.For high resolution images, please visit the specific product page at www.agrisera.com

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Depending upon abundance of a protein under investigation different sensitivity level of chemiluminescent reagent used for detection can be applied as compared in this 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 the affinity purified anti-PsbA primary antibody (AS05 084A) at a dilution of 1: 40 000 from 1 mg/ml stock.

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 in blocking reagent. Agrisera ECL SuperBright or Agrisera ECL Bright were used respectively, for detection. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 60 seconds, enhanced.