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**Product no** AS13 2659

## CCA1 | Circadian clock associated 1

## Product information

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|---|---|
| **Immunogen** | KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* CCA1 protein sequence, UniProt:P92973, TAIR:AT2G46830 |
| **Host** | Rabbit |
| **Clonality** | Polyclonal |
| **Purity** | Immunogen affinity purified serum in PBS pH 7.4. |
| **Format** | Lyophilized |
| **Quantity** | 50 µg |
| **Reconstitution** | For reconstitution add 50 µl of sterile water |
| **Storage** | Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube. |

## Application information

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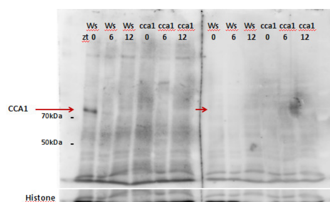
| **Recommended dilution** | 1: 500 (Co-IP), 1 : 500 (WB) |
| **Expected \| apparent MW** | 67 \| 80 kDa |
| **Confirmed reactivity** | *Arabidopsis thaliana* |
| **Not reactive in** | *Oryza sativa* |
| **Additional information** | **Important note about protein extraction**<br>Transcription factors are best isolated by freezing nucelar extract in liquid nitrogen after addition of extraction buffer, followed by thawing directly at 100°C heating block, not at RT. Centrifugation for 5 minutes will remove cell debris. Protein concenration is measure ad rest is frozen in liquid Nitrogen, in aliquots. Frozen samples are thawed at RT and loaded as soon as they are in liquid form, without heating. Such precautions are necessary to take to be able to detect a transcription factor using antibodies. |
| **Selected references** | Hung er al. (2018). The Arabidopsis LDL1/2-HDA6 histone modification complex is functionally associated with CCA1/LHY in regulation of circadian clock genes. Nucleic Acids Res. 2018 Aug 14. doi: 10.1093/nar/gky749. |

**application example**




A clear band for CCA1 was detected at about 80kDa after electrophoresis using a 8% polyacrylamid gel cast in a Biorad gel device with 15 wells and 1mm thickness. Nuclear extract of *Arabidopsis thaliana* Ws (Wasilewska), zt0 zt6 and lhy/cca1/toc1 zt0 was analyzed and protein concentration was equalized according to Bradford quantification to ~10µg proteins per lane after resuspending and heating to 100°C for 10 min in SDS loading buffer. Proteins were well separated on the PAGE, the gels were equilibrated for 10 min in blotting buffer of 29g/L Glycine, 5,9g/L Tris base with 20% MEOH. Proteins were transferred in a wet western transfer device from Biorad for 12 h on a PVDF membrane. >From 50µg/50µl stock solution the antibody was diluted 1:500 in 1X TBS and incubated with or without 10µg/ml of peptide for 1h at RT followed by two hours incubation on separated parts of the same blot. Washing the unspecific bound antibody from the blot with TBS 0.1% tween for one hour changing solutions twice after the incubation. Secondary antibody was goat anti-rabbit HRP conjugated (AS09 602) used at 1:5000 in TBS with 5% milk for two hours shaking at RT. With TBS Tween unspecific bond antibody was washed of for one hour changing solution twice. For the last Wash TBS without tween was used for about 10 minutes. As a substrate for the HRP,  the most sensitive chemiluminescent detection reagent was used in extreme low femtogram range. Pictures were taken with 10sec exposure and increment setting. After 3 pictures taken a clear band was detectable.

Courtesy of Dr. Mark Ruhl, Umeå Plant Science Centre, Sweden