

product **AS08 310**

**NR | Nitrate reductase, assimilatory**

### product information

<b>background</b>	Assimilatory nitrate reductase ( <b>NR</b> , EC.1.6.6.1) catalyses the reduction of nitrate to nitrite in the cytoplasm. Plants contain 2 forms of NR: NADH-NR (most common form in plants and algae, predominantly found in green tissues) and NAD(P)H-NR (uses NADH or NADPH as the electron donor, constitutively expressed in plants at a low level). NADH-NR is a homodimer of two identical subunits (100-115 kDa each, hold together by a Mo-cofactor) each of them coded by up to three genes (NR1-3, NIA1-NIA3).
<b>immunogen</b>	<u>KLH</u> -conjugated synthetic peptide derived from conserved domain in NADH-NR protein sequences including <i>A.thaliana</i> NR1 <a href="#">P11832</a> and NR2 <a href="#">P11035</a>
<b>antibody format</b>	rabbit; polyclonal; affinity purified serum; lyophilized
<b>quantity</b>	200 µg - for reconstitution add 100 µl of sterile water
<b>storage</b>	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.
<b>tested applications</b>	Western blot (WB)
<b>additional information</b>	to be added when available

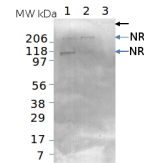
### application information

<b>recommended dilution</b>	1: 1000 (WB)
<b>expected   apparent MW</b>	103 kDa   117 kDa
<b>confirmed reactivity</b>	<i>Arabidopsis thaliana</i> , <i>Hordeum vulgare</i> , red alga <i>Gracilaria gracilis</i> , diatom <i>Thalassiosira</i> sp.
<b>predicted reactivity</b>	dicots including: <i>Glycine max</i> , <i>Lycopersicum esculentum</i> , <i>Nicotiana tabacum</i> , <i>Ricinus communis</i> , <i>Solanum tuberosum</i> , monocots including: <i>Oryza sativa</i> , <i>Zea mays</i> , moss: <i>Physcomitrella patens</i> ; <i>Chlamydomonas reinhardtii</i> , <i>Chlorella vulgaris</i> , <i>Dunaliella salina</i> , marine diatoms
<b>not reactive in</b>	no confirmed exceptions from predicted reactivity known in the moment
<b>additional information</b>	ECL based detection systems are advised to use since to low signal intensity can be obtained with BCIP/NBT system.  For working with diatom samples ECL Advance (GE Healthcare) or other more sensitive ECL detection reagent is recommended.

selected references | to be added when available

## application example - dicots

Detection of NR in protein extracts from leaves of *Columbia* (1) and NR knockout (2-3) mutants of *Arabidopsis thaliana*. Each lane was loaded with the protein contained in 2mg fresh weight leaf tissue (~15µg total protein). Mutants contained 56% (2) and 5% (3) of the NR activity of *Columbia*. Proteins were extracted in 0.1M Hepes pH 7.5, 1mM EDTA, 0.1% Triton X-100, 2 mM dithiothreitol, 0.7% Protease cocktail mix for plants (Sigma), and separated on 12% SDS-PAGE and blotted for 1h to nitrocellulose. Filters were blocked for 1h with 2% BSA in TBS-T (0.1% TWEEN 20) and probed with anti-NR antibodies AS08 310 in dilution 1:1000, 1h incubation time and secondary anti-rabbit (1: 4 000, 1 h) antibody (HRP conjugated, DAKO) in TBS-T containing 2% BSA. Antibody incubations were followed by three washings in TBS-T (15 min each). All steps were performed at RT with agitation. Signal was detected on blue light sensitive film (exposure time 1 min) with standard ECL (GE Healthcare). The image was captured with GS800 calibrated densitometer (BioRad, UK Ltd). Black arrow shows gel origin, blue arrows show bands of cross reactivity.

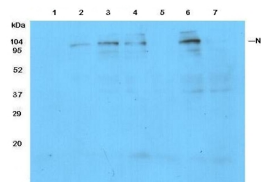


The mutant has 56% of the activity because NR is coded for by two genes and this sample is from a single knockout line.

Courtesy Dr. Alison Kingston-Smith and Teri Davies, Aberystwyth University

## application example - monocots

20 µl of *Hordeum vulgare* protein extract (equivalent to 2mg fresh weight) from (-N03+Dark) (1), (+N03+ Dark) (2), (+N03+24hr Light) (3), (+N03+ 24hr Light + Cytokinin) (4), (-N03+ Light) (5), (+N03+Light) (6), (+N03+ Light + Cycloheximide) (7). The barley plants were grown in the Dark or light 16/8 cycle for 6 days followed by 24hrs with or without Nitrate. Proteins from 7 days old plants were extracted in buffer containing 0.2M Phosphate buffer pH 7.5, 10% SDS, 5mM DTT and Pefabloc. The proteins were separated on 12% SDS-PAGE and transferred overnight to PVDF membrane. The Western Blot was treated for 1hr each with anti-NR (AS08 310) 1:1000 dilution and Goat anti Rabbit antibodies conjugated to Horse reddish peroxidase (GAR-HRP) 1:5000 dilution. Signal was detected using ECL kit (GE Health care).



Courtesy J. Mehroke and Dr. S. Singh, University of British Columbia