

product **AS08 372**

AtPex14p | peroxysomal marker

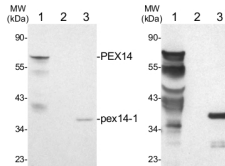
product information

background	Pex14p has a protein transporter activity and is involved in protein targeting into peroxisome. Submitted protein name: Genomic DNA, chromosome 5, P1 clone:MQB2
immunogen	recombinant, soluble N-terminal domain of <i>Arabidopsis thaliana</i> Pex14p (<u>Q9FE40</u>) that mediates PEX5 and PEX19 binding. The transmembrane and coiled-coil domains of PEX14 were replaced with a dual StrepII-His6 tag.
antibody format	rabbit polyclonal affinity purified serum lyophilized
quantity	200 µg for reconstitution add 100 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 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.
tested applications	western blot (WB)
additional information	Pex14p is prone to degradation.

application information

recommended dilution	1: 10 000 with standard ECL (WB)
expected apparent MW	55.5 75-65 kDa (depending upon the gel system)
confirmed reactivity	<i>Arabidopsis thaliana</i>
predicted reactivity	<i>Arabidopsis thaliana</i>
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
additional information	Antibodies will detect Pex14 protein in both light- and dark-grown seedlings grown on petri dishes and in rosette leaves of adult plants grown in soil.
selected references	To be added when available, antibodies released in November 2010.

application example



8-day-old light-grown *Arabidopsis thaliana* seedlings wild type (1), *pex14-2*, a T-DNA insertion allele in exon 1 of Pex14 (2), *pex14-1*, a nonsense allele midway through PEX14 (3) were ground with a pestle in a 1.5 mL tube dry ice (about 12 seedlings or enough to give ~20 μ L of tissue), and double volume 40 μ L of NuPAGE 2x loading buffer (Invitrogen). After centrifugation, 20 μ L of the supernatant was transferred to a fresh tube with 0.5 M DTT and boiled at 100°C for 5 minutes. Samples were loaded onto NuPAGE 10% Bis-Tris gels (Invitrogen) next to Cruz Markers (Santa Cruz Biotechnology). After electrophoresis, proteins were transferred for 30 minutes at 24 V to a Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech) using NuPAGE transfer buffer (Invitrogen). The blot was blocked for 1 h 4°C in 8% non-fat dry milk in TBS-T (blocking buffer), and incubated overnight with agitation at 4°C with primary antibodies (1:10 000 rabbit anti-PEX14, AS08 372, Agrisera, or 1:20 000 mouse anti-HSC70, SPA-817, StressGen Bioreagents) diluted in blocking buffer. The antibody solution was decanted and the blot was rinsed 5 min each with blocking buffer at 4°C. The blot was incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (Santa Cruz Biotechnology) 1:5 000 in blocking buffer for 4 h at 4°C with agitation. The blot was washed three times, for 5 min each, with TBS-T and developed for with LumiGLO reagent (Cell Signaling Technology) according to the manufacturer's instructions. Exposure time was 10 seconds (left panel) or 1 minute (right panel).

Courtesy Sarah Christensen and Dr. Bonnie Bartel, Rice University. USA.