Agrisera
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Product no AS08 300

1 | PEB (4x) | protein extraction buffer

Product information

Quantity 5 x 2 ml (4x stock) allows up to 75 isolations of plant material (using 500 µl 1x PEB for 100 mg fresh weight) or 190 isolations of algal material (using 200 µl 1x PEB for cell amounts corresponding to 4-10 µg total chlorophyll)

Storage Stable at RT for at least 1 month; short-term storage (6 monthss) at 4°C and long term storage (1 year or more) at -20°C.

Additional information **Buffer components (4x)**: contains ~ 40% v/v glycerol [$HOCH_2CH(OH)CH_2OH$], Tris-HCl [$NH_2C(CH_2OH)_3 \cdot HCl$] pH 8.5, LDS [$CH_3(CH_2)_{11}OSO_3Li$], EDTA [$(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$]

It is recommended to include a protease inhibitor (not supplied with this buffer) from a freshly made stock while preparing the ready-to-use 1x PSB. Please, note that some proteins which are aimed to be analyzed can be subjected to proteosomal degradation. Therefore, inhibitors as MG132 must be added.

PEB has been optimized for quantitative small-scale preparation of whole protein extracts from plant/algal tissue. Extraction using the procedure described below will result in maximum yield of proteins and diminish protein degradation and aggregation.

Extracts may be quantified using detergent (LDS) compatible methods and have been shown to give highly reproducible and quantitative results in subsequent SDS PAGE gel electrophoresis, Western Blotting, and immunoprecipitation.

PEB has been tested on a wide range of species and tissues from higher plants, mosses, lichens, algae, diatoms, dinoflagellates, and cyanobacteria.

Application information

Confirmed reactivity PEB has been tested on a wide range of species and tissues from Higher plants, Mosses, Llichens, Algae, Diatoms, Dinoflagellates, Cyanobacteria. Extracts may be quantified using detergent (LDS) compatible methods, and have been shown to give highly reproducible and quantitative results in subsequent SDS PAGE gel electrophoresis, Western blotting, and Immunoprecipitation (IP).

Most of Agrisera catalogue antibodies are tested on plant or algal samples extracted with this buffer. An example can be found here.

Selected references

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Pérez-López et al. (2020). Transcriptome Analysis Identifies Plasmodiophora brassicae Secondary Infection Effector Candidates. J Eukaryot Microbiol. 2020 Jan 11. doi: 10.1111/jeu.12784.
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Matsuo and Atsumi (2018). Xylosylation of proteins by expression of human xylosyltransferase 2 in plants. J Biosci Bioeng. 2018 Sep;126(3):371-378. doi: 10.1016/j.jbiosc.2018.03.013.
Brouwer et al. (2011) TheImpact ofLightIntensity onShade-InducedLeaf Senescence. Plant Cell Environ. Dec. 15 (ahead of print).
Kosawang et al. (2011) Hydrogen yield from a hydrogenase in Frankia R43 at different levels of the carbon source propionate. Journal of Environmental Management, Jan 26

Before you start
Prepare sufficient 1x PEB for all samples by diluting 4x stock in sterile, deionized water (the pH of your 1x PEB should be between 8.25 and 8.75). It is recommended to include a protease inhibitor (not supplied with this buffer) from a freshly made stock while preparing the ready-to-use 1x PEB to increase the yield of non-degraded protein in the extract. We recommend including 1:50 vol/vol from a freshly prepared 50x stock (in 1x PEB) to give the desired final concentration recommended by the manufacturer (e.g. 0.1 mg/ml for Pefabloc SC, Roche).

The total volume of 1x PEB required is dependent on the sample type and amount of tissue used: for 100 mg fresh plant tissue we recommend 500 µl 1x PEB; for algal samples (corresponding to 4-10 µg total chlorophyll) we recommend 200 µl 1x PEB. Keeping sample volumes in a range of 0.2-0.5 ml has been found to contribute to better extraction results, an upscale in volume is not recommended.

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Material preparation

Plant tissue: weigh and snap freeze in liquid nitrogen and store at -80°C until processing.
Algal cultures: centrifuge to form a pellet or collect on filters (e.g.GF/F or polycarbonate) and freeze at -80°C until processing.

Extraction

1	Grind frozen material in liquid N_2 in a pre-chilled mortar with a pestle to a fine powder and transfer to a 1.5 ml tube	Keep material cool at any time during grinding, avoid spillage
2	Add 1x PEB and immediately freeze sample in liquid N_2	500 µl for 100 mg plant tissue or 200 µl for cells corresponding to 4-10 µg total chlorophyll; keep tube upright to hold sample at the bottom of the tube
3	Carefully subject sample to sonication just until sample is thawn, re-freeze sample immediately in liquid N_2 to avoid heating	Optimal results will be obtained using a microtip sonicator (e.g. Branson Ultrasonics Model 450)at low settings of about 30%; waterbath sonicators may also be used though this may lead to slightly less reproducible protein recovery rates;
4	Repeat sonication step (3) depending on species, place on ice until all samples are processed	For higher plants 2-3 cycles, for cyanobacteria 3 cycles, for *Chlamydomonas* 2 cycles, for *Heterosigma, Thalassiosira* and *Trichodesmium* 1 cycle
5	Centrifuge your samples for 3 min at 10 000 x g to remove insoluble material and unbroken cells, the pellet should be white/light-grey	An intense green color of the pellet indicates that disruption was not optimal and extraction conditions need to be adjusted (e,g, improved grinding and/or repeated sonication steps) using a new sample
6	Transfer supernatant to new tube using a pipette, be careful not carry over debris	Expect ~400 µl supernatant for the plant and ~150 µl for cyanobacterial/algal samples; collecting supernatant with a pipette as 2 x 200 (or 2 x 75 µl) reduces the risk of disturbing the pelleted debris

Protein determination
Assay total protein content of recovered supernatant using a detergent compatible assay. Based on the amount and/or tissue of the species used you may expect a protein content of 1.5-6 µg/µl.

Storage
Protein extracts may be stored for 24 hrs at +4°C or up to 6/12 month at -20°C/-80°C. We recommend to aliquot samples. Re-freezing protein samples may induce degradation/aggregation.

Loading on a gel
A freshly prepared reducing agent should be added (e.g. Dithiotreitol, final concentration 50 mM) to the volume prepared for loading. Heat at 70°C for 5 min, briefly spin down and load on a gel. Protein loads of 0.5-5 µg/lane should be sufficient for most Western Blot applications.