Product: **Protein Solubilisation Buffer, PSB** (4x)

Product no: AS08 300

Agrisera
Antibodies for plant sciences

Product Information

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

Storage instructions: stable at RT for at least 1 month; short term storage (6 month) at 4°C or long term storage (1 year) at -20°C

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.

Advantages

PSB 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.

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

Background

PSB is an extraction buffer for disruption and solubilisation of total protein from plant tissue and algal cells. The use of the anionic detergent LDS together with the recommended procedure (combination of sonication and freeze/thaw cycles) has been shown to increase the amount of solubilised and non-degraded proteins when compared to other methods of cell disruption (see reference).
The estimated hands-on time for the recommended procedure is 20-30 minutes for 1-2 samples. Expected yields will be 1.5-6 µg/µl total protein (recovered from standard procedure) depending on the starting material.

Before you start

Prepare sufficient 1x PSB for all samples by diluting 4x stock (the pH of your 1x PSB 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 PSB 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 PSB) 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 PSB required is dependent on the sample type and amount of tissue used: for 100 mg fresh plant tissue we recommend 500 µl 1x PSB; for algal samples (corresponding to 4-10 µg total chlorophyll) we recommend 200 µl 1x PSB. 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 PSB 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.

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