

OREGIN_Summary procedure for Brassica seed protein extraction & separation_RRES 2008

<i>OREGIN Standard Operating Procedure</i>	
Purpose	Extract and separate seed proteins into constituent fragments of different size
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Method for analysis of *Brassica* seed proteins by SDS-PAGE

Extraction

Total soluble proteins are extracted from 5 *Brassica spp.* seed (~20 mg) as described by Shewry et al. (1995), with the exception that bromophenol blue was not added to the extraction buffer. The use of a Hamilton syringe to remove the supernatant prevents oil contamination.

Quantification

Protein extracts are quantified using the Biorad Protein microassay (cat# 500-0006), according to the manufacturer's instructions. Bovine serum albumin (BSA) (Sigma A7030) is used for the standard curve.

Protein Separation

Protein extracts (3 µg total protein per sample) are separated by SDS-PAGE according to the method of Laemmli (1970) on a Biorad Protean II system, with 15% resolving and 5% stacking gels, respectively, run at 200 V for 55 min. Benchmark Prestained Ladder (Invitrogen 10748-010) is used as the MW standard.

Staining Gel

Gel is fixed, stained and destained according to the manufacturer's instructions for Brilliant Blue G-colloidal concentrate (Sigma B2025).

Analysing Gel

Gel and Kodak Control Scale T-14 strip are scanned using the HP Scanjet 3970 and saved as 256 greyscale .tif files. Proteins are quantified using Phoretix™ 1D software (Nonlinear Dynamics Ltd, Newcastle-upon-Tyne, UK).

References

Shewry, P.R., Tatham, A.S., Fido, R.J. (1995). Separation of plant proteins by electrophoresis. In: *Methods in Molecular Biology—Plant Gene Transfer and Expression Protocols*, **49**: 423–437. Jones, H. (ed.). Humana Press, Totowa, NJ.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680–685.