LABORATORY FOR HEALTH, OMICS AND PATHWAY EXPLORATION

DPPH Radical Scavenging Assay for Antioxidant Activity

A method reference for DPPH-based antioxidant screening, blank correction, controls and concentration-response interpretation.

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HOPE Lab student reference: This assay is included among the methods used in relevant HOPE Lab research and training. The document supports convenient access to the method's scientific basis, controls and interpretation; it does not replace supervised laboratory instruction.

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Method boundary: This guide summarizes the assay design and data interpretation; it does not provide reagent-preparation quantities or bench instructions. Follow the laboratory's chemical risk assessment and approved SOP.

Purpose and scope

The DPPH radical-scavenging assay is a colorimetric method used to estimate the in-vitro radical-scavenging capacity of an extract or prepared sample. It is commonly reported as a preliminary antioxidant assay and should not be equated with antioxidant efficacy in a biological system.

Principle

The stable DPPH radical has a strong visible absorbance band near 515-517 nm. Reaction with a reducing or radical-scavenging component decreases the absorbance. Response depends on concentration, solvent, reaction kinetics, sample colour and the selected endpoint; therefore, matched blanks and a concentration-response design are important.

Materials and measurement categories

- A validated DPPH reagent system and a compatible solvent selected under the laboratory's chemical-safety procedure.
- Prepared test extracts or samples with documented concentration and solvent composition.
- A spectrophotometer or microplate reader capable of the selected visible wavelength.
- A calibration reference such as Trolox or ascorbic acid and appropriate sample/solvent blanks.

Controls and blanks

Control	Purpose
DPPH reagent control	Provides the reference absorbance for the radical solution without test sample.
Solvent blank	Documents solvent/background absorbance.
Sample blank (without DPPH)	Corrects for intrinsic colour or turbidity from the sample.
Positive antioxidant reference	Checks assay responsiveness and supports calibration or comparison.
Concentration series	Reveals whether the response is concentration-dependent and supports EC50/IC50 estimation where appropriate.

Analysis framework

A blank-corrected calculation may be expressed as:

$$\text{DPPH scavenging (\%)} = [1 - (A_{\text{sample+DPPH}} - A_{\text{sample blank}}) / (A_{\text{DPPH control}} - A_{\text{solvent blank}})] \times 100$$

Define each absorbance term explicitly in the methods. Report the reference antioxidant, tested concentration range, reaction endpoint, wavelength, solvent composition and blank-correction approach. When suitable, estimate EC50 or IC50 from a fitted concentration-response relationship and report the model and uncertainty.

Interpretation and limitations

- DPPH is a chemical radical-scavenging screen; it does not measure all antioxidant mechanisms or predict in-vivo effects.
- Coloured or turbid samples can interfere with absorbance and require sample-specific blanks.
- Different solvents and reaction times can change apparent activity; compare samples only under matched conditions.
- Use independent preparations and technical replicates, and report both raw and blank-corrected data where feasible.

References and further reading

1. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5

2. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry. 2005;53(10):4290-4302. doi:10.1021/jf0502698

Document status: Version 1.0. This reference should be checked against current literature, institutional requirements and the approved local method before use.