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Product guide: Nitroreductase

JM Johnson
Matthey



The enzymes in this kit have been selected for their great activity on a wide selection of substrates and to accommodate the different synthetic needs of our customers.

Substrates accepted by the NR enzymes include aromatic and heteroaromatic nitro compounds^[1].

An outstanding feature of this catalytic system is the exquisite chemoselectivity.

Substrates containing halogens or other functional group that could be lost or reduced upon chemical nitroreduction (i.e. O-benzyl, C-C double bonds, nitriles) are reduced by the nitroreductase vanadium catalytic system only at the nitro-moiety, leaving untouched the other functional groups.

^[1] Synergistic nitroreductase/vanadium catalysis enables chemoselective nitro reductions to anilines in the absence of hydrogen gas. Curr. Res.Chem Biol. 2022, 2, 100026.

Kit description

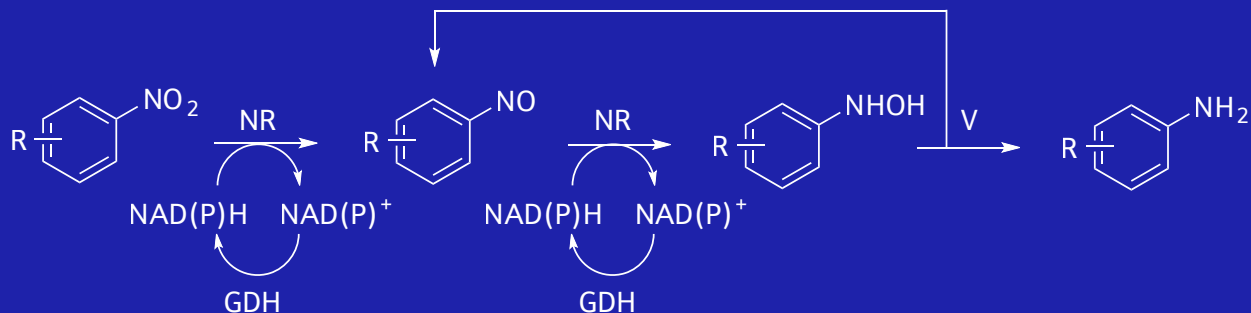
This kit contains 8 nitroreductase (NR) enzymes for the reduction of nitroaromatic compounds to their corresponding anilines.

The enzymes in this kit are recombinantly expressed from bacterial sources and include enzymes from thermophilic microorganisms and engineered enzymes.

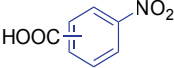
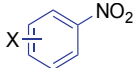
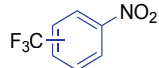
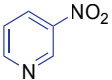
NRs need a nicotinamide cofactor (preferably NADPH) for reducing the nitro group. To be able to use this cofactor in catalytic amounts, the oxidised cofactor has to be continuously reduced through what is called cofactor regeneration.

The enzymes accept preferably NADPH cofactor and its regeneration can be achieved by adding a cofactor regeneration enzyme and its hydride donating substrate (e.g. glucose dehydrogenase, aka GDH, and D-glucose, or alcohol dehydrogenase, aka ADH, and 2-propanol).

In addition, to obtain complete reduction of the nitro group to the corresponding aniline, it is necessary to add catalytic amounts of vanadium (e.g. V2O5, or NH4VO3) that catalyses the disproportionation of the hydroxylamine intermediate.



Enzyme overview

Enzyme	Cofactor Preference				
NR-5	NADP ⁺	++	++	+	++
NR-14	NADP ⁺	++	++	+	++
NR-20	NADP ⁺	+	-	++	++
NR-23	NADP ⁺	+	-	++	++
NR-24	NADP ⁺	++	++	+	++
NR-36	NADP ⁺	++	+	++	++
NR-48	NADP ⁺	++	++	-	++
NR-55	NADP ⁺	++	-	++	++

Key

 ++ High activity  + Moderate activity X = Halogen group

Reaction setup and work-up

1. Weigh 5 mg NR into an Eppendorf tube or reaction vial.
2. In a separate container prepare the Reaction Mix dissolving 7.6 mg NADP⁺ (1 mM), in 9 mL Potassium Phosphate Buffer (250 mM, pH 7) and add 180.2 mg D-glucose (100 mM), 10 mg GDH-101 (1 mg/mL) and 3.6 mg of V2O5 (2 mM).
3. Add 450 µL Reaction Mix to the enzyme powder.
4. Add 50 µL of the substrate of choice from a 200 mM stock (final substrate concentration in the reaction 20 mM) prepared in toluene.
5. Incubate the reaction at 30-35 °C for 18 hours in a thermostated shaker or with magnetic stirring.
6. Quench the reaction by adding 1 mL acetonitrile to the mixture followed by vigorous mixing, on vortex for 30 seconds, and centrifugation, through a benchtop centrifuge at 13,000 rpm for 2 minutes, before transferring the clarified supernatant to a glass vial for HPLC analysis.

Useful tips

- Recommended pH for NR biocatalysed nitroreduction is 7. Higher pH will increase the formation of unwanted dimeric side-products while lower pH is known to slow down the disproportionation step. For an initial screening, Potassium Phosphate Buffer at pH 7 is recommended. To prepare 500 mL of 250 mM Potassium Phosphate buffer mix 13.39 g K₂HPO₄ and 6.55 g KH₂PO₄ in milliQ or distilled water.
- For analysis, it is recommended to use HPLC, rather than GC. GC analysis is known to cause artefacts with the substrate, product and intermediates of the reaction, leading to misrepresentative results.
- Using a co-solvent, such as toluene, can help solubilise the substrate and facilitate dispensing it into the reaction. Other recommended co-solvents are: DMSO, TBME, n-heptane, isoamylacetate. Solvents that can have detrimental effect on the enzyme stability comprise methanol, ethanol, 2-methyl THF. In general, up to 50% (v/v) co-solvents can be tolerated and 5-10% (v/v) co-solvent is recommended. Alternatively, substrates can be added neat to the reaction, though it can affect the conversion and selectivity if highly insoluble in water.



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