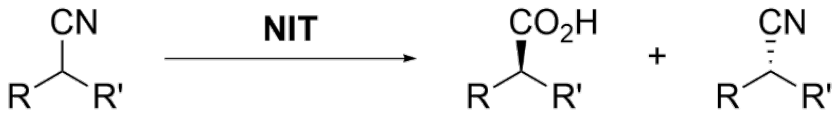
Codex® Nitrilase Screening Kits

Screening Protocol

Reaction of Interest



Codex® Nitrilase Screening Kit General Information

1. The Codex® Nitrilase Screening Kit is a useful tool to quickly determine the feasibility of converting nitriles into carboxylic acids. The 12 enzymes included in this kit exhibit a broad substrate range, diverse stereoselectivity, improved solvent stability and can operate on a wide range of temperature and pHs.

2. The recommended storage temperature for the enzyme powders is-20 °C.

3. Gram quantities of the nitrilases in this kit are available from stock for any follow up work.

Codex® Nitrilase Screening Kit Contents

Item	Enzyme	Amount
1	NIT-102	250 mg
2	NIT-103	250 mg
3	NIT-104	250 mg
4	NIT-105	250 mg
5	NIT-106	250 mg
6	NIT-111	250 mg
7	NIT-P1-118	250 mg
8	NIT-P1-120	250 mg
9	NIT-P1-121	250 mg
10	NIT-P1-122	250 mg
11	NIT-P1-126	250 mg
12	NIT-P1-130	250 mg
13	NIT-RXN BUFFER (Nitrilase Reaction Buffer Mix)	7.25 g

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1. Reconstitute 0.725 g of the NIT-RXN BUFFER (Nitrilase Reaction Buffer Mix) by adding 80 mL deionized water. Reconstituted buffer contains 50 mM potassium phosphate, 2 mM DTT, 1 mM EDTA, pH 7.5. Buffer should be reconstituted fresh on the day of the reaction.

2. For each NIT enzyme, dissolve 10 mg of nitrile with 5 mL Nitrilase Reaction Buffer Mix. Add 10 mg NIT enzyme to start the reaction. It is OK if the nitrile does not completely dissolve. If the nitrile is very insoluble in water, a solution of the nitrile (in a solvent such as DMSO or methanol) can be added to the reaction mixture. We recommend that the solvent does not exceed 5% of the total volume of the reaction solution for screening purposes.

3. Incubate the reactions with agitation at 30 °C overnight. Aliquots can be removed and quenched at various intervals to monitor the reaction if desired.

Work-Up and Analysis

1. Depending on the anticipated method of analysis, follow one of the protocols below:

    a. If using reversed phase HPLC for analysis, the reaction can be quenched by adding 5 mL acetonitrile to the reaction and mixing well to dissolve any insoluble substrate and product. More acetonitrile can be added if this is not enough to solubilize the substrate and product.

    b. If using normal phase HPLC or GC for analysis, add 1 mL of 6 M HCl to quench reaction and extract into 5 mL of isopropyl acetate or MTBE.

2. Centrifuge the reactions at ~4000 rpm for 10 min to separate the phases or to sediment any precipitated protein. If a centrifuge is not available an extractive workup can be done and the phases can be allowed to separate unaided, or the reaction can be filtered through a 0.2-micron filter.

3. Transfer the aqueous acetonitrile or the organic phase from each reaction to a shallow-well plate or vials for analysis of selectivity and/or conversion by preferred method of analysis.

For further information or any questions, please contact us at: sales@codexis.com.

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