

SET OF COLORIMETRIC ASSAYS FOR ENZYME TESTS: SCREENING AND ACTIVITY DETERMINATION

SUMMARY

Enzyme screening and activity test platforms at laboratory and industrial scales require the utilization of appropriate methods. Colorimetric methods are among the most useful ones for such purposes as they are based on the utilization of easy-to-use particular commercially available substrates, some of which may have interest at industrial settings, to target enzymes coding activities of interest.

DESCRIPTION OF THE PRODUCT

The MAGICPAH Consortium gets ready a set of ready-to-use colorimetric methods based on the following enzyme-substrates tandems:

Esterases and lipases :

- α-Naphtyl acetate
- (+)-Methyl (R)-2-chloropropionate
- (+)-methyl D-lactate
- (-)-Methyl L-lactate
- (1R)-(-)-Menthyl acetate
- (1R)-(-)-Neomenthyl acetate
- (1S)-(+)-Menthyl acetate
- (1S)-(+)-Neomenthyl acetate
- (R)-(-)-Glycidyl butyrate
- (S)-(+)-Glycidyl butyrate
- 2-Bromoethyl acetate
- Butyl acetate
- Ethyl (R)-(-)-3-hydroxybutyrate
- Ethyl (S)-(-)-4-chloro-3-hydroxybutyrate
- Ethyl 2-chloropropionate
- Ethyl 2-methylacetoacetate
- Ethyl 4-chloroacetoacetate
- Ethyl 4-hydroxy-3-methoxycinnamate
- Ethyl acetate
- Ethyl bromoacetate
- Ethyl caprate
- Ethyl chloroacetate
- Ethyl fluoroacetate
- Ethyl hexanoate
- Ethyl iodoacetate
- Ethyl octanoate

- Ethyl propionate
- Ethyl propionylacetate
- Ethyl tribromoacetate
- Ethyl trifluoroacetate
- Ethyl-2-bromopropionate
- Ethyl-3-bromopropionate
- Ethyl-4-bromobutyrate
- Ethyl-α-isobromobutyrate
- Ethyl-trans-cinnamate
- Glycerol trihexanoate
- Glycine ethyl ester
- Isobutyl acetate
- Isobutyl cinnamate
- Isopropenyl acetate
- Methyl (R)-(+)-3-bromo-2-methylpropionate
- Methyl (S)-(+)-3-hydroxy-2-methylpropionate
- Methyl (S)-(+)-3-hydroxybutyrate
- Methyl 2,2-dimethyl-3-hydroxypropionate
- Methyl 2-hydroxyisobutyrate
- Methyl 4-(hydroxymethyl)benzoate
- Methyl acetoacetate
- Methyl benzoate
- Methyl bromoacetate
- Methyl butyrate
- Methyl chloroacetate
- Methyl cinnamate
- Methyl glycolate
- Methyl propionate
- Methyl pyruvate
- Methyl α-bromophenylacetate
- Methyl trans-cinnamate
- Methyl-(R)-(-)mandelate
- Methyl-(S)-(+)-mandelate
- Methyl-2-chloro-3-hydroxypropionate
- Methyl-2-chloropropionate
- Methyl-3-bromopropionate
- Phenethyl cinnamate
- Phenyl acetate
- p-Nitrophenyl acetate
- p-Nitrophenyl butyrate
- p-Nitrophenyl propionate
- Propyl acetate
- tert-Butyl 3-hydroxypropionate
- tert-Butyl cinnamate
- Triacetin



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Tributylin
Tricaprin
Tricaprylin
Trilaurin
Tripropionin
Vinyl acetate
Vinyl benzoate
Vinyl butyrate
Vinyl cinnamate
Vinyl crotonoate
Vinyl decanoate
Vinyl laurate
Vinyl methacrylate
Vinyl pivalate
Vinyl propionate

C-C meta-cleavage product hydrolases:

2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate
2-Hydroxy-6-oxohepta-2,4-dienoate

Aromatic ring extradiol dioxygenases:

Catechol
3-Methyl catechol
4-Chloro catechol
4-Methyl catechol
2,3-Dihydroxybiphenyl

Aromatic ring mono- and dioxygenases

Indol - indigo

Laccases:

Remazol Brilliant Blue
Guaiacol
2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)

Glycosyl hydrolases:

*p*NP- β -acetylglucuronide
*p*NP- α -arabinofuranoside
*p*NP- β -arabinopyranoside
*p*NP- β -D-cellobioside
*p*NP- β -D-galactopyranoside
*p*NP- β -D-glucopyranoside
*p*NP- α -D-maltohexaoside
*p*NP- α -D-maltopentaoside
*p*NP- β -D-mannopyranoside
*p*NP- β -fucoside

*p*NP- α -fucoside
*p*NP- α -glucopyranoside
*p*NP- β -glucuronide
*p*NP- β -lactopyranoside
*p*NP- α -L-galactopyranoside
*p*NP- α -L-rhamnopyranoside
*p*NP- α -maltoside
*p*NP- α -mannopyranoside
*p*NP- α -xylopyranoside
*p*NP- β -xylopyranoside

* *p*NP: *p*-nitrophenyl

INNOVATIONS AND ADVANTAGES OFFERED

- The assays can be used for: (1) screening or selection systems to be applied in liquid or solid assays, for example, using clone libraries; and (2) colorimetric assays for enzyme characterization and determination of substrate specificity and kinetic parameters.
- The assays require a low substrate concentration, typically ranging from μ g to mg per ml.

POTENTIAL AREA OF UTILIZATION

The enzyme activities covered by the ready-to-use colorimetric methods have received considerable attention because they are among the most important groups of biocatalysts for biotechnological applications, including: (1) production of chiral synthons; (2) activation of inert molecules; and (3) esterification and transesterification reactions, to cite some.

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