

Cutinase Fs

EC 3.1.1.74

Description: Enzyme preparation for the hydrolysis of cutin and other esters. Cutin (polyester of hydroxy and hydroxy-epoxy fatty acid) is cleaved into the corresponding monomers.

Application: Splitting of cutin; organic synthesis (Tab. 1)

Origin: *Fusarium solani*, expressed in *Arxula adenivorans*

Activity: 10.000 U/g (pH 7,0; 37°C; Glycerintributytrat as substrate)

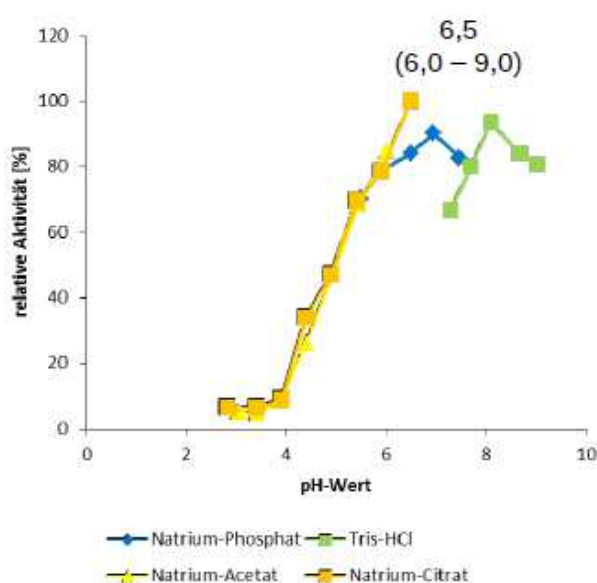
Reaction parameters: pH optimum: 6 - 9 active in the area pH 5 - 10
Temperatur optimum: 25 - 50°C active in the area 20 - 60°C

Order-No.: 2460

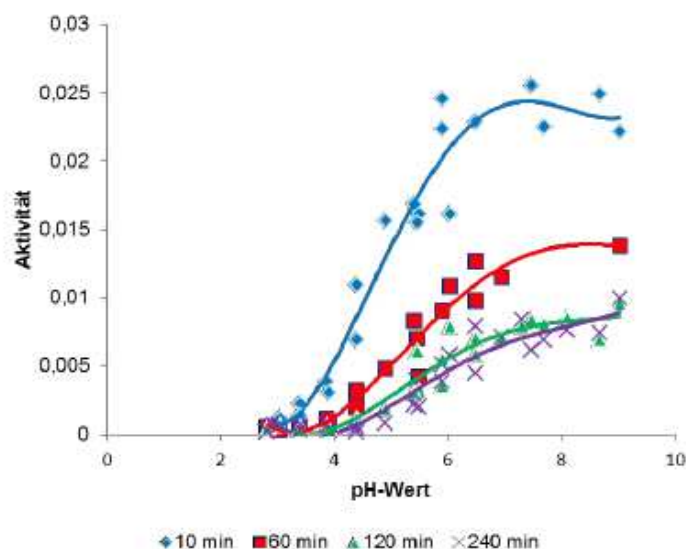
Packaging: partially purified lyophilisate

Storage: at -20°C

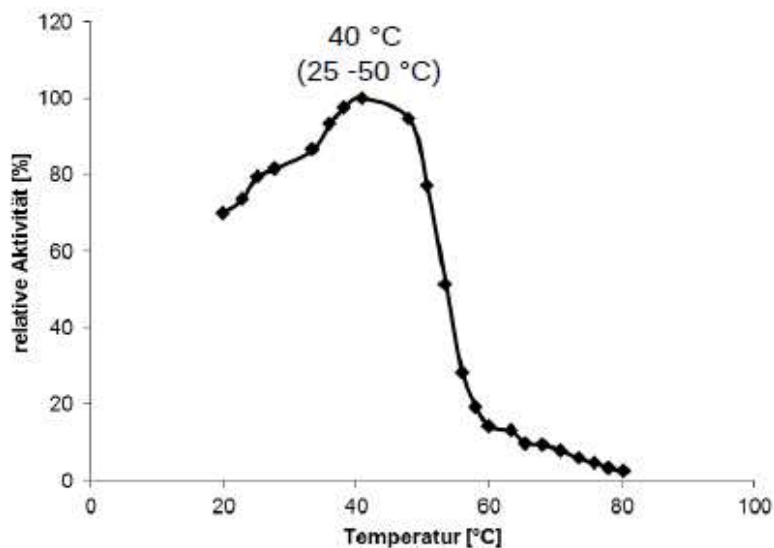
Stability: 12 months under the above stated conditions



III. 1: pH dependence of cutinase Fs



III. 2: pH stability of cutinase Fs



III. 3: Temperature dependence of cutinase Fs

Tab. 1: Use of cutinases in bio catalysis

(due to Carvalho et al., 1998b)

Reaction	Substrate	Enzyme preparations/ reaction	Reference.
Hydrolysis	triolein	Reverse Micelles AOT/ isooctane	Melo et al., 1995b
		triolein/ water	Flipsen et al., 1996
	tricaprylin	Immobilization on zeolites	Gonçalves et al., 1996a
		Calcium alginate	Gonçalves et al., 1995
		Covalent bonding on porous silicate	Gonçalves et al., 1996b
	p-nitrophenyl valerate	Micelles with SDS/ Triton X100	Pocalyko and Tallman, 1998
	p-nitrophenyl palmitate	Immobilization on dextran and silica-derivatives	Gonçalves et al., 1998a
Methyl-,ethyl-, Propyl propionate	Gas/ solid phase system	Lamare et al., 1997	
Ester synthesis	oleic acid + hexanol	Reverse micelles AOT/ isooctane	Sebastião et al., 1993, Sebastião et al., 1992
	caprylic acid + butanol	Organic solvents	Sarazin et al., 1992, Sarazin et al., 1995
	caprylic acid + butanol	Organic solvents	Sarazin et al., 1992, Sarazin et al., 1995
	butanoic acid + 2-butanol	Phosphatidylcholine/ isooctane, reverse micelles	Pinto-Sousa et al., 1994
	oleic acid + glycerin	Organic solvents	Melo et al., 1995a

	hexanoic acid + hexanol	CTAB, Reverse micelles	Cunnah et al., 1996
		Immobilization on Accurel EP 100	Sereti et al., 1997
	butanoic acid + hexanol	Immobilization on Accurel EP 100	Sjursnes et al., 1998
	lauric acid + pentanol	Reverse micelles AOT/ isooctane	Papadimitriou et al., 1996
Transesterification	methyl propionate + propanol	Gas/ solid phase system	Lamare and Legoy, 1995, Lamare et al., 1997
	butyl acetate + hexanol	Reverse micelles AOT/ isooctane	Carvalho et.al 1997a, Carvalho et al., 1998a
		Reverse micelles CTAB/ isooctane	Cunnah et al., 1996
		Immobilization on zeolites	Serralha et al., 1998

Literatur

Carvalho, C.M.L., Serralheiro, M.L.M., Cabral, J.M.S. and Aires-Barros, M. R. (1997a). Application of factorial design to the study of transesterification reactions using cutinase in AOT-reversed micelles. *Enzyme and Microbiological Technology* 21: 117-123.

Carvalho, C.M.L., Cabral, J.M.S. and Aires-Barros, M. (1998a) Kinetics and modelling of transesterification reactions catalysed by cutinase in AOT reversed micelles. *Journal of Molecular Catalysis B- Enzymatic* 5: 361-365.

Carvalho, C. M. L., Aires-Barros, M. R. and Cabral, J. M. S. (1998b). Cutinase structure, function and biocatalytic applications *EJB Electronic Journal of Biotechnology*, 1: 160-173.

Cunnah, P.J., Aires-Barros, M.R. and Cabral, J.M.S. (1996). Esterification and transesterification catalysed by cutinase in reverse micelles of CTAB for the synthesis of short chain esters. *Biocatalysis and Biotransformation* 14:125-146.

Flipsen, J.A.C., van der Hijden, H.T.W.M., Egmond, M.R. and Verheij, H.M. (1996) Action of cutinase at the triolein/water interface. Characterisation of interfacial effects during lipid hydrolysis using the oil-drop tensiometer as a tool to lipase kinetics. *Chemistry and Physics of Lipids* 84:105-115.

Gonçalves, A.P.V., Cabral, J.M.S. and Aires-Barros, M.R. (1995) Immobilization of a recombinant cutinase by entrapment and by covalent binding. *Applied Biochemistry and Biotechnology* 60:217-228.

Gonçalves, A.P.V., Lopes, J.M., Lemos, F., Ramôa Ribeiro, F., Prazeres, D.M.F., Cabral, J.M.S. and Aires-Barros, M.R.(1996a) Zeolites as supports for enzymatic hydrolysis reactions. Comparative study of several zeolites. *Journal of Molecular Catalysis B- Enzymatic* 1:53-60.

Gonçalves, A.M., Schacht, E., Cabral, J.M.S. and Gil, M.H. (1996b) Stability studies of a recombinant cutinase immobilised on an inorganic support. In: *Proceedings of III Congreso Ibérico de Biotecnología*, F.F. Polanco, P.G. Encina, G.G. Benito and M.M. P. Miranda, Universidad de Valladolid, pp. 551-552.

Gonçalves, A.M., Schacht, E., Matthijs, G., Aires-Barros, M.R., Cabral, J.M.S., Gil, M.H. (1998a) Stability studies of a recombinant cutinase immobilized to dextran and derivatized silica supports. *Enzyme and Microbial Technology*. In press.

Lamare, S. and Legoy, M.D. (1995) Working at controlled water activity in a continuous process: the gas/solid system as a solution. *Biotechnology and Bioengineering* 45:387-397.

Lamare, S., Lortie, R. and Legoy, M.D. (1997) Kinetic studies of *Fusarium solani pisi* cutinase used in a gas/solid system: transesterification and hydrolysis reactions. *Biotechnology and Bioengineering* 56:1-7.

Melo, E.P., Ivanova, M.G., Aires-Barros, M.R., Cabral, J.M.S. and Verger, R. (1995a) Glyceride synthesis catalyzed by cutinase using the monomolecular film technique. *Biochemistry* 34:1615-1621.

Melo, E.P., Aires-Barros, M.R., Cabral, and J.M.S. (1995b) Triglyceride hydrolysis and stability of a recombinant cutinase from *Fusarium solani* in AOT-*iso*-octane reversed micelles. *Appl. Biochem. Biotechnol.* 50:45-56.

Papadimitriou, V., Xenakis, A., Cazianis, C.T., Stamatis, H., Egmond, M. and Kolisis, F.N. (1996) EPR studies of cutinase in microemulsions *Annals NewYork Academy of Sciences* 799:275-280.

Pinto-Sousa, A.M., Cabral, J.M.S. and Aires-Barros, M.R. (1994) Ester synthesis by a recombinant cutinase in reversed micelles of a natural phospholipid. *Biocatalysis* 9: 169-179.

Pocalyko, D.J. and Tallman, M. (1998) Effects of amphipaths on the activity and stability of *Fusarium solani pisi* cutinase. *Enzyme and Microbial Technology* 22: 647- 651.

Sarazin, C., Goethals, G., Séguin, J.P., Legoy, M.D. and Barbotin, J.N. (1992) Usefulness of NMR methods for assaying cutinase catalysed synthesis of ester in organic media. In: *Biocatalysis in Non-Conventional Media*, J. Tramper, M.H. Vermue, H.H. Beftink and U. von Stockar. Elsevier Science Publishers B.V., 1992, pp. 23-29.

Sarazin, C., Ergan, F., Séguin, J.P., Goethals, G., Legoy M.D. and Barbotin J.N. (1995) NMR on-line monitoring of esterification catalyzed by cutinase. *Biotechnology and Bioengineering* 51:636-644.

Sebastião, M. J., Cabral, J.M.S., and Aires-Barros, M.R. (1992) Synthesis of fatty acid esters by a recombinant cutinase in reversed micelles. In: *Biocatalysis in Non- Conventional Media*, J. Tramper, M.H. Vermue, H.H. Beftink and U. von Stockar. Elsevier Science Publishers B.V., 1992, pp. 719-724.

Sebastião, M. J., Cabral, J.M.S., and Aires-Barros, M.R. (1993) Synthesis of fatty acid esters by a recombinant cutinase in reversed micelles. *Biotechnology and Bioengineering* 42:326-332.

Serralha, F.N., Lopes, J.M., Lemos, F., Prazeres, D.M.F., Aires-Barros, M.R. Cabral, J.M.S., and Ribeiro, F. R. (1998) Zeolites as supports for an enzymatic alcoholysis reaction . *Journal of Molecular Catalysis B- Enzymatic* 4:303-311.

Sereti, V., Stamatis, H. and Kolisis, F.N. (1997) Improved stability and reactivity of *Fusarium solani* cutinase in supercritical CO₂. *Biotechnology Techniques* 11:661-665.

Sjursnes, B., Valente, C. and Halling, P. (1998) Interactions between water and medium effects on enzymes in organic media: kinetics of cutinase catalysed esterification with mutual solvation of reactants. *Biotechnology and Bioengineering* In press.