

ENZYMES

Cholesterol Oxidase (Recombinant)

ORIGIN Original gene expressed in *Streptomyces lividans*

CAT# RECO-70-1221
EC# 1.1.3.6

SPECIFICATIONS

Appearance Bright yellow to brown free flowing powder
Activity >25 U/mg powder at 25°C
Specific Activity >25 U/mg protein at 25°C
Contaminants **Catalase** <1%
Glucose Oxidase <0.01%
Uricase <0.01%

ASSAY PRINCIPLE

Cholesterol Oxidase (CO) catalyses the following reaction:



UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will catalyse the oxidation of 1.0 micromole of cholesterol per minute at 25°C under the standard assay method conditions. Refer to Table 1 for guidance on factors to adjust units according to temperature of assay.

TABLE 1: TEMPERATURE FACTORS FOR UNIT CONVERSION

ASSAY TEMPERATURE	FACTOR RELATIVE TO 25° RESULT
25°C	1.00
30°C	1.12
37°C	1.24
45°C	1.28

Note: Temperature can influence the level of available oxygen in the reaction mixture.

APPLICATION

Cholesterol Oxidase can be used in combination with Cholesterol Esterase (in test strips or other clinical chemistry formats) to determine the level of cholesterol in blood.

CHARACTERISTICS

This recombinant grade of CO is highly purified and formulated without additives. Its main characteristics are as follows:

Molecular Weight (by SDS Page):	58kD
K_m value (using Eadie-Hofstee):	7 x 10 ⁻⁶ M (Cholesterol)
Optimum pH (Fig. 1):	pH 6.5 to 7.5
Optimum Temperature (Fig. 2):	45°C
Stable pH Range (Fig. 3):	pH 4.5 to 9.0 (25°C for 20 hrs)
Thermal Stability (Fig. 4):	Stable at 60°C and below (pH 7.0 for 15 mins)

SUBSTRATE SPECIFICITY

Substrate specificity was tested in-house by replacing cholesterol with alternative substrates in the CO assay i.e. at 0.2mM concentration.

SUBSTRATE	% OF CHOLESTEROL ACTIVITY	SUBSTRATE	% OF CHOLESTEROL ACTIVITY
Cholesterol	100%	Cholesteryl Linoleate	0.9%
Pregnenolone	116%	Estradiol	0.2%
β-Cholestanol	86%	Cholecalciferol	0.1%
Stigmasterol	55%	Androsterone	<0.1%
Ergosterol	35%	Sodium Cholate	<0.1%

FIGURE 1: OPTIMUM pH

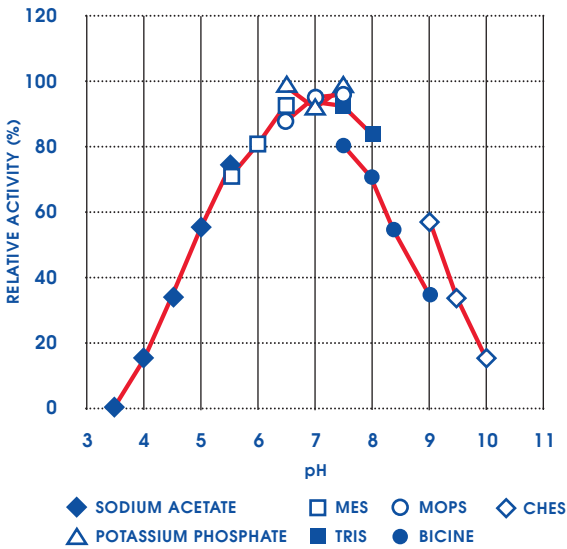


FIGURE 2: OPTIMUM TEMPERATURE

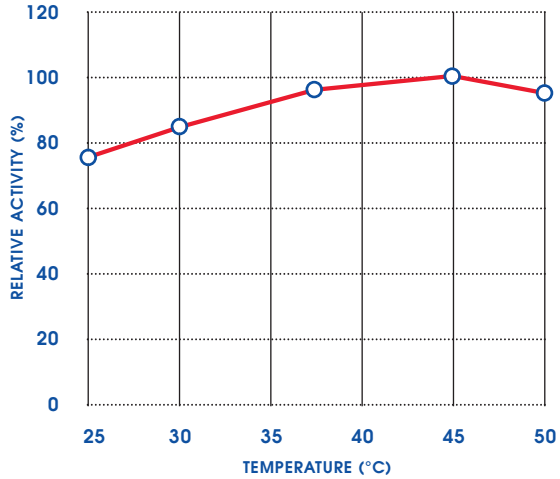


FIGURE 3: pH STABILITY (25°C FOR 20 HOURS)

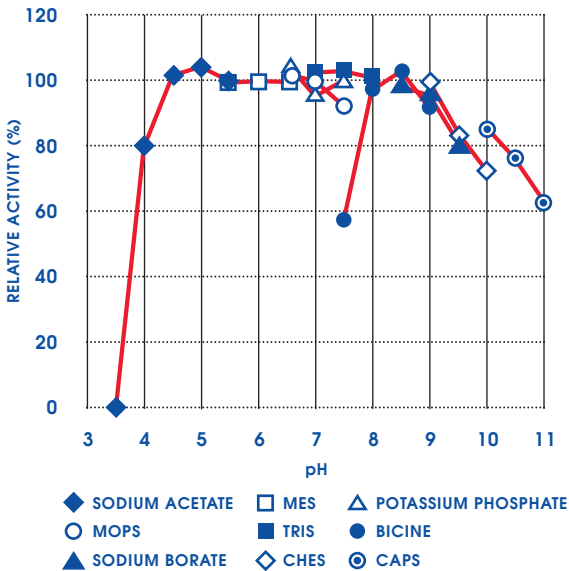
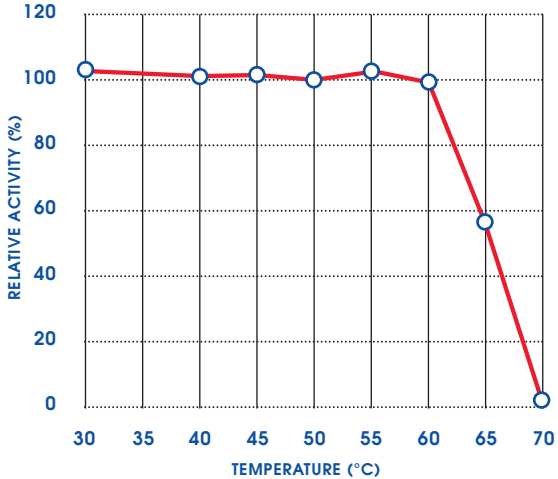


FIGURE 4: THERMAL STABILITY (pH 7.0 FOR 15 MINUTES)



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