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Process Change Notification

Affected Product	Microbial (bacterial) Transglutaminase
Product number	T001
Reason	Increasing demand of Microbial (bacterial) transglutaminase (T001)
Change description	<p>Microbial (bacterial) transglutaminase is recombinantly produced in the inactive zymogenic form (Pro-MTG) in <i>E. coli</i> in a shaker flask fermentation. To achieve the production of larger amounts of Pro-MTG, a stirred tank fermentation process was developed. For this purpose, the expression plasmid was changed. Essentially, an expression plasmid carrying a kanamycin-resistance gene is used instead of the former expression plasmid, which contained a β-lactamase-gene.</p> <p>The fermentation medium was changed. The medium contains lactose, the only material of animal origin within the MTG production process is. The lactose used is certified by the supplier to be TSE and BSE free. No other animal derived media components are used in the stirred tank fermentation protocol. No other material of animal origin is used for MTG production and purification nor added to the product.</p>
Conformity assessment	<p>A thorough conformity assessment was performed, in order to confirm the quality of the MTG purified from recombinant <i>E. coli</i> cells produced according to the novel fermentation protocol.</p> <p>The novel fermentation protocol showed a stable and reproducible fermentation course in 3 independent runs.</p> <p>Cells derived from 3 independent fermentation runs were used for the production of 3 lots of Microbial (bacterial transglutaminase (T001). Analysis was performed according the standard QC-procedure for T001. Moreover, a sample of each lot was analysed by mass spectrometry to confirm the correct size of MTG.</p> <p>The results are summarized in Table 1 on the next page</p>
Result	<p>The transfer of the Pro-MTG production protocol from shaker flask to stirred tank fermenter, as described above, did not have an impact on the product quality of microbial transglutaminase (T001). Microbial (bacterial) transglutaminase (T001) lots 3420aT001, 3620aT001 and 3720aT001 fulfill all QC requirements and are released.</p>
Measure	<p>Future lots of T001 are produced according to the new stirred tank fermentation protocol. T001 customers will be notified about the change.</p>

Table 1: Conformity assessment of 3 independent lots of Microbial (bacterial) transglutaminase (T001), produced from recombinant *E. coli* cells obtained by 3 individual stirred tank fermentations. The packaging unit was 25 U per vial.

	QC-Specification	lot 3420aT001	lot 3620aT001	lot 3720aT001
Specific activity [U/mg]	> 25 U/mg	31.6	31.8	33.5
Purity HPLC	>98%	100%	100%	100%
SDS-PAGE	One single band at 38 kDa			
Mass [Da] (ESI MS analysis)	38333	38333	38333	38333

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