

# Chapter 12

## Measure of Peptidoglycan Hydrolase Activity

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### Abstract

Most gene clusters encoding multiprotein complexes of the bacterial cell envelope, such as conjugation and secretion systems, Type IV pili, and flagella, bear a gene encoding an enzyme with peptidoglycan hydrolase activity. These enzymes are usually glycoside hydrolases that cleave the glycan chains of the peptidoglycan. Their activities are spatially controlled to avoid cell lysis and to create localized rearrangement of the cell wall. This is assured by interaction with the structural subunits of the apparatus. Here we describe protocols to test the peptidoglycan hydrolase activity of these proteins in vitro and in solution.

**Key words** Cell wall, Localized degradation, Peptidoglycan, Lytic transglycosylase, Remazol blue

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### 1 Introduction

The peptidoglycan is a mesh-like structure that provides the shape and protection against external pressure to bacterial cells. It is composed of glycan chains resulting from the polymerization of *N*-acetylmuramic acid (MurNAc)-*N*-acetylglucosamine (GlcNAc) disaccharides. These chains are linked by peptide stems that differ from one species to another. With pores of approximately 2 nm, the cell wall constitutes a physical barrier for the passage of macromolecules and for the assembly of cell-envelope-spanning complexes [1–3]. Most trans-envelope multiprotein machineries therefore have evolved dedicated enzymes that locally degrade the cell wall to provide sufficient space for their assembly and insertion without compromising the bacterial shape and survival [3, 4]. These enzymes usually cleave the  $\beta$ -1,4 bond between the *N*-acetylmuramic acid and the *N*-acetylglucosamine of the glycan chains and form nonreducing 1,6-anhydromuropeptides characteristic of lytic transglycosylases (LTGs) [4–7]. Genes encoding these enzymes are found associated in Type III secretion, Type IV secretion, or flagellum gene clusters [3, 4, 6]. The best-studied specialized LTGs are FlgJ and SltF, which are associated with flagellar assembly [8–10], and EtgA, VirB1, and TagX/MltE,