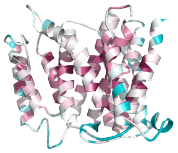
MMP9 Enzyme Assay

Protocol



The following is a brief description of activation reagents for collagenases. These are intended to be used only as guidelines. Please refer to the CHEMICON **MMP Activation Chart** for further information on enzyme structural and functional comparisons and related references.

1 **APMA** (amino-phenyl mercuric acetate) is widely used for the activation of latent pro-collagenase (1).
2 **Trypsin** activates both latent pro-collagenase and collagenase bound by inhibitors such as α2M and low molecular weight collagenase inhibitors. (2,3). However, soybean trypsin inhibitor (SBTI) must be added before assaying collagenase activity.
3 **KSCN** or **KI** is used to activate collagenase bound by inhibitors such as α2M (4). These reagents are useful to denature collagenase inhibitors in sample specimens prior to activating procollagenase by APMA (5).
4 **DTT** (Dithiothreitol) and **Iodoacetamide** have been reported to activate collagenases bound by TIMPSs (6). However, these reagents may inactivate collagenase by reducing the S-S bonds and by alkylating the glutamic acid in the active site. Therefore, the limitations of this method must be taken into consideration.

References:

1 Sellers A., Cartwright E., Murphy G., Reynolds , JJ, Biochem J. 163: 303-307 (1977)
2 Shinkai et al., J. Biochem. 81: 261-263 (1977)
3 Shinkai H., Nagai Y. J. Biochem. 81: 1261-1263 (1977)
4 Abe S., Nagai Y., Biochim. Biophys, Acta 278: 125-132 (1972)
5 Murawaki Y. et al., J. Hepatology, 18: 328-334 (1993)
6 Rajabi MR., Dean DD., Woessner JF., Obstet Gynecol. 159: 971-976 (1988)

APMA Solutions [p-Aminophenylmercuric acetate] APMA will not dissolve in weak base or water. It will dissolve in 100% DMSO, however DMSO can influence enzyme activity so most tend not to use it.

Dissolve 3.5mg in 1-2 mls of 0.1M NaOH. At 3.5mg/ml this should be 10mM. Before use, most tend to neutralize the high base by diluting in it neutral buffer. Thus the 10mM stock is diluted 4X with reaction buffer. A typical buffer is Tris-Triton-Calcium (TTC). {50mM Tris-HCL pH 7.5, 1mM CaCl2, 0.05% triton X-100}. Standard PBS will also work as long as it has calcium. MMPs are quite flexible for buffers; pH should be near neutral for best activity (though MMPs will degrade more quickly at neutral pHs). If necessary adjust pH to 7.0-7.5 (using 0.1N HCL). This diluted stock is approximately 2.5mM. APMA concentrations from 0.5-3.0mM are typical. This 2.5 mM solution can be used to directly dilute MMP enzymes for activation. Or this stock may also be diluted further with TTC to achieve any range of APMA. For instance, routinely, we take 1 part this to 9 parts MMP sample and incubate it at 37°C for 30 minutes for conditioned media samples. This final solution can be used directly without dialyzing away the APMA. Activation times will vary depending upon the samples. MMP-2 generally requires short activation times, others like MMP-1 & 9 often require longer times (3-5 hours are typical), but optimal activation times must be determined empirically.

NOTE: APMA is not stable, thus diluted stock should be made fresh. 10mM stocks are kept only for 1 week at 4°C. APMA solutions higher than 20mM tend to precipitate out when added directly to reactions, thus pre-diluting as detailed above is recommended.

1. Sellers A., Cartwright E., Murphy G., Reynolds , JJ, Biochem J. 163: 303-307 (1977).