

# A Novel and Specific Assay for the Measurement of Matrix Metalloproteinase-14 (Membrane Type Metalloproteinase MT1-MMP) Activity in Biological Samples.

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## Introduction

The matrix metalloproteinases (MMPs) are a family of over 20 enzymes that cleave the various components of the extracellular matrix (ECM)<sup>(1)</sup>. These enzymes are associated with a variety of normal and pathological conditions that involve matrix degradation and remodelling. The tissue destruction that occurs in diseases such as periodontitis, rheumatoid arthritis and tumour cell invasion during metastasis are mediated by members of the MMP family.

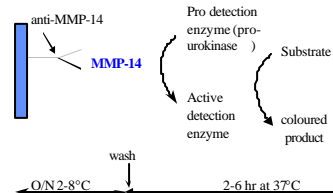
MMP-14 (MT1-MMP) is the first cloned and isolated membrane-type MMP that activates proMMP-2 and pro-MMP-13<sup>(2,3)</sup>. In addition to the activation of MMP-2 and MMP-13, MMP-14 is able to directly degrade extracellular matrix proteins such as interstitial collagens, fibronectin, vitronectin and laminin.

MMP-14 has been detected in carcinomas of lung, cervix, stomach, colon and brain (mostly based on immunohistochemistry and mRNA determinations). MMP-14 is expressed at the cell surface and therefore solubilisation of the enzyme from the membrane is needed before its activity can be measured.

We have developed a novel, specific, simple, immunocapture activity assay that allows the measurement of MMP-14 activity in biological samples.

## Method

The MMP-14 activity assay is based on the Quickzyme<sup>™</sup> technology platform that employs a modified pro-urokinase<sup>(4)</sup>. It employs a specific antibody to MMP-14 coated onto a 96-well microplate. Membrane-bound MMP-14, solubilised in a buffer containing Triton<sup>™</sup> X-100, is captured from the cell (or tissue homogenate) during overnight incubation at 4°C. After a wash step, the level of immobilised MMP-14 is measured by detecting at 405nm the cleavage by the activated urokinase of a chromogenic substrate (see Figure 1).



**Figure 1.** Schematic representation of the MMP-14 activity assay.

Active MMP-14 can be measured in the range 0.125 – 32ng/ml for a 2 hour incubation (0.2 – 4ng/ml using a 6 hour incubation period). The sensitivity of the assay is 0.7ng/ml using a 2-hour incubation.

Both the pro and the active forms of MMP-14 can be measured using this assay. However, data obtained with this assay supports previous reports that only active MMP-14 is present at the cell surface of various cell types<sup>(2,5)</sup>.

## Results

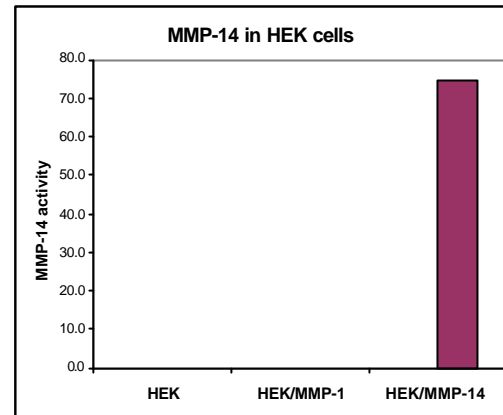
The assay recognises both the pro and active forms of MMP-14. Other MMPs have been assayed for cross-reactivity (see Table 1).

Enzyme/pro-enzyme	% Cross-reactivity
Active MMP-14	100
Active MMP-1	3.6
Active MMP-2	1.5
Active MMP-3	0.2
Active MMP-9	0.4
Pro MMP-8	0*
Pro MMP-13	0*

**Table 1.** Cross-reactivity of the MMP-14 activity assay with other MMPs.

\* Pro MMP-8 and Pro MMP-13 were added to cell cultures before extraction. All other cross-reactants were tested using purified MMPs that were activated with APMA prior to being assayed.

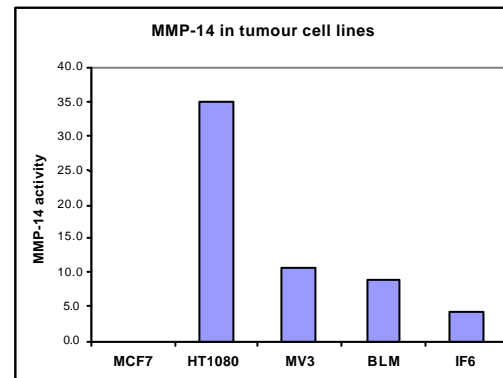
Conditioned culture medium and cellular extracts from HEK cells either untransfected or transfected with MMP-1 or MMP-14 were analysed for MMP-14 activity. No enzyme activity was detected in any of the conditioned culture media. High levels of MMP-14 were observed in the cellular extracts of MMP-14 transfected cells. However, no activity was detectable in the cellular extracts obtained from MMP-1 transfected cells (see Figure 2).



**Figure 2.** Expression of MMP-14 in HEK cells transfected with MMP-1 and MMP-14.

Cells, either untransfected or transfected with an MMP-1 or MMP-14 expression vector, were cultured for 48 hr. Culture medium was then collected and cells were solubilised and analysed for MMP-14 activity. (A unit of enzyme activity is defined as:  $DA_{405} \times 1000/t$ , where  $t$  is the incubation time).

Cellular extracts from various tumour cell lines (melanoma, fibrosarcoma) were analysed for MMP-14 activity - see Figure 3.



**Figure 3.** Expression of MMP-14 in various tumour cell lines.

MCF7, HT1080, MV3, BLM and IF6 cells were cultured and extracts analysed for MMP-14 activity.

(A unit of enzyme activity is defined as in Figure 2).

Other sample types that have been shown to contain detectable MMP-14 activity include lung tissue homogenates, synovial fibroblast extracts, breast cancer tissue homogenates and synovial tissue homogenates obtained from patients with rheumatoid arthritis (data not shown).

The MMP-14 activity assay that has been developed provides a convenient means to quantify this important membrane associated metalloproteinase in biological samples.

## CONCLUSIONS

- A simple, specific and precise quantitative assay for the determination of membrane-bound and membrane-released (shed) MMP-14 activity in biological samples has been developed.
- The activity assay can be applied to many sample types.

## References

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