

GE Healthcare

Amersham

Matrix

Metalloproteinase-14

(MMP-14), Biotrak

Activity Assay System

Product Booklet

Code: RPN2637



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QuickZyme™ MMP-14 Activity Assay is based on a technology developed in collaboration with TNO Prevention and Health, Gaubius Laboratory, PO Box 2215, 2301CE Leiden, The Netherlands.

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## 2. Handling

### 2.1. Safety warnings and precautions

**Warning: For research use**

**only.** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see materials safety data sheets and/or safety statements for specific advice).

### 2.2. Storage

Store at -15°C to -30°C

### 2.3. Expiry

The expiry date is stated on the package and will normally be at least 4 weeks from the date of despatch.

## 3. Components

### **Microplate**

The plate contains 12 x 8 well strips coated with anti-MMP14. Ready for use.

### **Assay buffer**

Bottle contains 5 ml of a Tris-HCl buffer concentrate which when diluted gives 50 mM Tris-HCl buffer pH 7.6, containing 1.5 mM Sodium Chloride, 0.5 mM Calcium Chloride, 1  $\mu$ M Zinc Chloride, Heparin, 1 unit/ml and 0.01% (v/v) Brij™ 35.

### **Extraction buffer**

Bottle contains 10 ml of a Tris-HCl buffer concentrate which when diluted gives 50 mM Tris-HCl buffer pH 7.6, containing 1.5 mM Sodium Chloride, 0.5 mM Calcium Chloride, 1  $\mu$ M Zinc Chloride, 0.01% (v/v) Brij 35 and 0.25% (v/v) Triton™ X-100.

### **Standard**

Tube contains 64 ng of lyophilized human pro MMP-14. On reconstitution this gives a concentration of 64 ng/ml pro MMP-14 in 50 mM Tris-HCl buffer pH 7.6 containing 1.5 mM Sodium Chloride, 0.5 mM Calcium Chloride, 1  $\mu$ M Zinc Chloride, 0.01% (v/v) Brij 35 and 0.25% (v/v) Triton X-100.

### **Detection enzyme**

Tube contains 100  $\mu$ l of concentrated solution of modified urokinase in 50 mM Tris HCl buffer pH 7.6 containing 1.5 mM Sodium Chloride, 0.5 mM Calcium Chloride, 1  $\mu$ M Zinc Chloride, Heparin 1 unit/ml and 0.01% (v/v) Brij 35.

### **Substrate**

Bottle contains lyophilized S-2444™ peptide substrate which on reconstitution gives a ready to use solution of S-2444 peptide substrate in 50 mM Tris-HCl buffer pH 7.6 containing 1.5 mM Sodium

Chloride, 0.5 mM Calcium Chloride, 1  $\mu$ M Zinc Chloride, Heparin 1 unit/ml and 0.01% (v/v) Brij 35.

**Wash buffer**

Bottle contains 12.5 ml of Phosphate buffer concentrate which when diluted gives a 0.01 M Sodium Phosphate buffer pH 7.0 containing 0.05% Tween™ 20.

## 4. Other materials required

### Materials and equipment

The following materials and equipment are required but not supplied:

- Pipettes or pipetting equipment with disposable polypropylene tips, (5  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l, 500  $\mu$ l, 1 ml and 5 ml).
- Disposable polypropylene test tubes.
- Glass measuring cylinders (50 ml, 100 ml and 500 ml).
- Distilled or deionized water.
- Microplate incubator at 37°C.
- Microplate shaker.
- Spectrophotometric plate reader capable of measuring at 405 nm.
- Refrigerator at 2–8°C.
- Automatic plate washer or wash bottle.

## 5. Critical parameters

The following points are critical:

- The assay buffer concentrate, extraction buffer, anti-MMP-14 coated microplate and wash buffer should be allowed to equilibrate to 20–27°C before preparation.
- It is important that all the wells are washed thoroughly and uniformly. If using an automatic washer, check operation of heads before starting. If washing by hand, use a wash bottle and ensure that all wells are completely filled at each wash.
- Use only coated wells from the same reagent batch for each assay.
- The incubation temperatures are critical. Sample capture should be performed at 2–8°C, but all other incubations are performed at 37°C.
- Preparation of working standards and addition of standards to microplate should be performed using polypropylene tips.
- Once thawed out, samples, standards, detection enzyme and substrate should be kept at 2–8°C, prior to performing the assay.
- Incubation times must be carried out exactly. If more than one plate is being assayed, each plate must be timed individually.
- A separate standard curve must be run on each plate.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents.
- Avoid handling the tops of wells both before and after filling.
- Keep the wells covered with lids except when adding reagents and reading.
- Standards and samples should be assayed in duplicate.
- The total dispensing time for each plate should not exceed 20 minutes.

## 6. Description

- Specific for MMP-14
- Non-radioactive
- Microplate based assay
- Precise and accurate measurement
- Applicable to complex biological samples
- Ready to use detection enzyme and substrate

The Biotrak™ MMP-14 activity assay system from GE Healthcare provides a simple, specific and precise quantitative determination of active MMP-14 in tissue culture and biological samples.

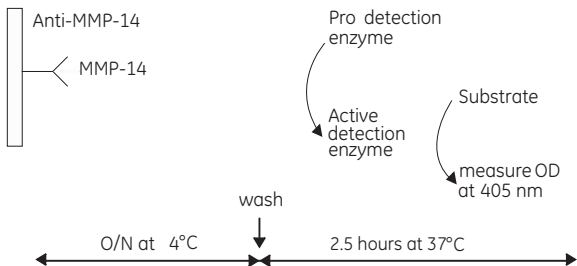
Active MMP-14 may be measured in the range 0.125 to 32 ng/ml and the sensitivity of the assay is 0.7 ng/ml for a 2 hour incubation.

The assay uses the pro form of a detection enzyme that can be activated by captured active MMP-14, into an active detection enzyme, through a single proteolytic event. The natural activation sequence in the pro detection enzyme has been replaced using protein engineering, with an artificial sequence recognized by specific matrix metalloproteinases. MMP activated detection enzyme can then be measured using a specific chromogenic peptide substrate (figure 1, page 10). Anti MMP-14 antibody is immobilized on to microplate. Standards and samples are incubated in the plate and any MMP-14 present is bound to the immobilized antibody. Washing and aspiration remove other components of the sample. Endogenous levels of free active MMP-14 in a sample can be detected. The standard is pro MMP-14. MMP-14 in both standards and samples becomes activated without further treatment. Active MMP-14 is detected through activation of the modified pro detection enzyme and the subsequent cleavage of its chromogenic peptide substrate. The resultant colour is read at 405 nm in a microplate spectrophotometer. The concentration of active MMP-14 in a sample

is determined by interpolation from a standard curve.

Each pack contains reagents for 96 determinations. This allows the construction of a standard curve plus the measurement of 41 samples in duplicate.

The Biotrak MMP-14 activity assay system has been specifically designed for research purposes.



**Figure 1.** Protocol for MMP-14 activity assay

## 7. Specimen collection and sample preparation

The Biotrak MMP-14 activity assay system from GE Healthcare has been tested with two types of samples for which representative procedures are described for guidance. It remains the responsibility of the investigator to validate the chosen procedure.

### **Tissue samples**

Users are advised to carefully validate any extraction procedure employed. The following method is described for the preparation of tissue samples from RA synovial tissue and is for general guidance only.

1. Grind the tissue.
2. Add 20–50 µl of extraction buffer per mg of ground tissue. (This may vary with tissue type).
3. Incubate for 15 minutes at 4°C.
4. Centrifuge at 2000 x g for 10 minutes at 4°C.
5. Assay the supernatant for MMP-14. A dilution of at least 1 in 3 is recommended.

### **Cell culture**

1. Culture cells in 24 well plates.
2. Remove the media and replace with 250 µl of extraction buffer per cm<sup>2</sup>.
3. Incubate at 4°C for 15 minutes.
4. Assay the supernatant for MMP-14. A dilution of at least 1 in 10 is recommended.

## 8. ELISA protocol

### 8.1. Reagent preparation

The assay buffer concentrate, extraction buffer, anti-MMP-14 coated microplate and the wash buffer should be allowed to equilibrate to 20–27°C before preparation. The other assay components should only be removed from storage just before use.

Either distilled or deionized water may be used for buffer preparation. The detection enzyme is ready for use once thawed but should be kept at 2–8°C prior to use. The microplate is ready for use when equilibrated to 20–27°C. The assay involves an overnight incubation.

#### **Day 1**

##### **Extraction buffer**

1. Transfer the contents of the bottle to a 100 ml measuring cylinder by repeat washing with distilled water.
2. Adjust the final volume to 100 ml with distilled water and mix thoroughly.
3. Place on ice for at least 30 minutes before use.

##### **Standard**

1. See standard vial for reconstitution volume.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Store on ice until required.

#### **Day 2**

##### **Assay buffer**

1. Transfer the contents of the bottle to a 50 ml measuring cylinder by repeat washing with distilled water.
2. Adjust the final volume to 50 ml with distilled water and mix thoroughly.

**Note: Solution may be cloudy on storage. This will disappear on dilution and does not affect the performance of the assay.**

### **Wash buffer**

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

**Note: Solution may be cloudy on storage. This will disappear on dilution and does not affect the performance of the assay.**

### **Detection enzyme**

1. Allow the tube containing the detection enzyme to thaw before use.
2. Store on ice until required.

### **Substrate**

1. Add 5.1 ml of assay buffer to the bottle and replace the stopper.
2. Gently mix until the contents are completely dissolved.

Vigorous agitation and foaming should be avoided.

3. Store on ice until required.

### **Detection reagent**

This reagent should be prepared only immediately prior to addition to the wells.

1. For every 9 wells take 10  $\mu$ l of the detection enzyme concentrate and add to 500  $\mu$ l of the reconstituted substrate.
2. If using the whole plate add 100  $\mu$ l of the detection enzyme concentrate to the reconstituted substrate.
3. Mix gently but thoroughly.
4. 50  $\mu$ l of detection reagent is added to each well during the assay protocol (see page 16).

Once reconstituted the assay buffer, extraction buffer and wash buffer should be stored at 2–8°C, and all other components, at -15°C to -30°C. All components should be reused within 4 weeks.

## 8.2. Assay range 1–32 ng/ml (for higher endogenous MMP-14 levels)

**Note: It is important to use a clean polypropylene pipette tip for each dilution. Working standards should be freshly prepared immediately prior to use in each assay and not reused.**

1. Label 6 polypropylene tubes for 1, 2, 4, 8, 16 and 32 ng/ml and place on ice.
2. Pipette 250 µl of cold extraction buffer into each tube.
3. Pipette 250 µl of the stock standard (64 ng/ml) into the 32 ng/ml tube.
4. Vortex mix.
5. Pipette 250 µl from the 32 ng/ml tube into the 16 ng/ml tube.
6. Vortex mix.
7. Repeat this doubling dilution step successively with the remaining tubes.
8. 100 µl aliquots from each serial dilution will give rise to 6 standard levels of MMP-14 ranging from 1 to 32 ng/ml.

**Note:** Stock standard at 64 ng/ml is not part of the standard curve. This may be stored at -15°C to -30°C if running only a partial plate.

### Assay protocol

1. Prepare the reagents as described in 'reagent preparation'.
2. Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required. Place on ice.

3. Pipette 100 ml of each standard into the appropriate wells, using a clean polypropylene pipette tip for each standard. Into the zero standard wells pipette 100  $\mu$ l cold extraction buffer.
4. Pipette 100  $\mu$ l of unknown sample into the appropriate wells.
5. Cover the plate with the lid provided, shake for 20 seconds and incubate at 2–8°C overnight.
6. Aspirate and wash all wells 4 times with wash buffer ensuring that the wells are completely filled and emptied at each wash.
7. Blot the plate on tissue paper ensuring any residual volume is removed during the blotting procedure.
8. Prepare the detection reagent as described in the reagent preparation section.
9. Pipette 50  $\mu$ l of assay buffer into all wells.
10. Briefly vortex the detection reagent to ensure thorough mixing.
11. Pipette 50  $\mu$ l of the detection reagent into all wells.
12. Shake the plate for 20 seconds.
13. Read the plate at 405 nm to obtain a  $t_0$  value.
14. Cover the plate with the lid provided and incubate at 37°C for 2.5 hours.
15. Shake the plate for 20 seconds.
16. Read the plate at 405 nm.

**Note:** The detection incubation can be continued for longer if required. Users who find their  $OD_{405}$  values to be lower than those quoted in the typical data section can do this to increase their optical densities accordingly.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	S	S	S	S	S	S	S	S	S	S
B	1	1	S	S	S	S	S	S	S	S	S	S
C	2	2	S	S	S	S	S	S	S	S	S	S
D	4	4	S	S	S	S	S	S	S	S	S	S
E	8	8	S	S	S	S	S	S	S	S	S	S
F	16	16	S	S	S	S	S	S	S	S	S	S
G	32	32	S	S	S	S	S	S	S	S	S	S
H	S	S	S	S	S	S	S	S	S	S	S	S

**Figure 2.** Recommended positioning of standard (1–32 ng/ml) and sample (S) wells

### 8.3. Assay range 0.125–4 ng/ml (for lower endogenous MMP-14 levels)

**Note:** It is important to use a clean polypropylene pipette tip for each dilution. Working standards should be freshly prepared immediately prior to use in each assay and not reused.

1. Label 9 polypropylene tubes for 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 ng/ml and place on ice.
2. Pipette 250  $\mu$ l of cold extraction buffer into each tube.
3. Pipette 250  $\mu$ l of the stock standard (64 ng/ml) into the 32 ng/ml tube.
4. Vortex mix.
5. Pipette 250  $\mu$ l from the 32 ng/ml tube into the 16 ng/ml tube.

6. Vortex mix.
7. Repeat this doubling dilution step successively with the remaining tubes.
8. Discard the 8, 16 and 32 ng/ml standards leaving 6 standard levels of MMP-14 ranging from 0.125 to 4 ng/ml.

**Note:** Stock standard at 64 ng/ml is not part of the standard curve. This may be stored at -15°C to -30°C if running only a partial plate.

### **Assay protocol**

1. Prepare the reagents as described in 'reagent preparation'.
2. Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required. Place on ice.
3. Pipette 100  $\mu$ l of each standard into the appropriate wells, using a clean polypropylene pipette tip for each standard. Into the zero standard wells pipette 100  $\mu$ l cold extraction buffer.
4. Pipette 100  $\mu$ l of unknown sample into the appropriate wells.
5. Cover the plate with the lid provided, shake for 20 seconds and incubate at 2–8°C overnight.
6. Aspirate and wash all wells 4 times with wash buffer ensuring that the wells are completely filled and emptied at each wash.
7. Blot the plate on tissue paper ensuring any residual volume is removed during the blotting procedure.
8. Prepare the detection reagent as described in the reagent preparation section.
9. Pipette 50  $\mu$ l of assay buffer into all wells.
10. Briefly vortex the detection reagent to ensure thorough mixing.
11. Pipette 50  $\mu$ l of the detection reagent into all wells.
12. Shake the plate for 20 seconds.
13. Read the plate at 405 nm to obtain a  $t_0$  value.

14. Cover the plate with the lid provided and incubate at 37°C for 6 hours.

15. Shake the plate for 20 seconds.

16. Read the plate at 405 nm.

**Note:** The detection incubation can be continued for longer if required. Users who find their OD<sub>405</sub> values to be lower than those quoted in the typical data section can do this to increase their optical densities accordingly.

For assays giving results with typical ODs, the longer incubation time will increase the assay sensitivity. However, standards may show signs of saturation upon prolonged incubation.

## 8.4. Data processing

### Calculation of results

The calculation is illustrated using representative data.

The assay data for the standard curve should be similar to that shown in table 1.

As MMP-14 activity is directly proportional to the generation of color through the cleavage of S-2444, it can be represented by the rate of change of absorbance at 405 nm, i.e.  $\delta\text{Abs}_{405}/h^2$ , where h is the incubation time in hours. The absorbance change is linear with respect to the square of the duration of incubation, indicating that the conditions for a parabolic assay rate were met.

The final data is actually multiplied by 1000 so as to be able to plot whole numbers on the graph. Hence the data is expressed as  $\delta\text{Abs}_{405}/h^2 \times 1000$ .

### Example calculation

The data obtained at t=0 is intended as a reference point on which the activity rate calculations are based. The absorbance<sub>405</sub> values for the standards at t=0, from our experience, are always the same

as those for the blank, when the curve is read. Hence, for simplicity, these values are omitted from the calculation.

The example calculation shown below is for the 32 ng/ml standard at a 2.5 hour detection incubation period. Rate of change of MMP activity should be expressed as:

$$\frac{\text{Abs}_{t=2.5} - \text{Abs}_{t=0} \times 1000}{h^2}$$

For a 2.5 hour incubation period the raw data in table 1 would therefore be plotted as shown in table 2.

However, as  $\text{Abs}_{t=0}$  is equivalent to zero, the calculations can be expressed as

$$(\delta\text{Abs}_{t=2.5}/h^2) \times 1000$$

#### **For a 2.5 hour detection incubation**

Mean absorbance<sub>405</sub> for 32 ng/ml standard = 0.377

Mean absorbance<sub>405</sub> for 0 ng/ml standard (blank) = 0.077

$$\delta\text{Absorbance}_{405} = 0.300$$

For a 2.5 hour incubation period:

$$\delta\text{Absorbance}_{405}/h^2 = \frac{0.300}{6.25}$$

$$= 0.048$$

$$\delta\text{Absorbance}_{405}/h^2 \times 1000 = 0.048 \times 1000$$
$$= 48$$

### Typical assay data

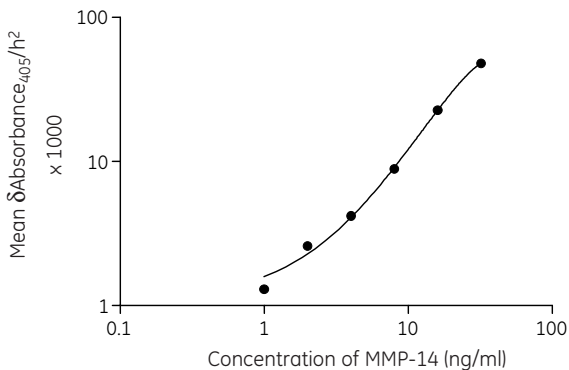
**Table 1.** Typical assay data for a standard curve (2.5 h incubation)

MMP-14 (ng/ml)	Absorbance <sub>405</sub>	Mean absorbance <sub>405</sub>
32	0.367	0.377
	0.387	
16	0.209	0.219
	0.228	
8	0.133	0.133
	0.133	
4	0.100	0.103
	0.106	
2	0.093	0.093
	0.093	
1	0.084	0.085
	0.086	
0	0.076	0.077
	0.079	

**Table 2.** Raw data expressed as  $\delta\text{Absorbance}_{405}/h^2 \times 1000$

MMP-14 ( ng/ml)	Mean $\delta\text{Absorbance}_{405}/h^2 \times 1000$
32	48
16	22.7
8	8.9
4	4.2
2	2.6
1	1.3
0	0

A standard curve is generated by plotting  $\delta\text{Absorbance}_{405}/h^2 \times 1000$  (y axis) against ng/ml standard (x axis). The standard curve shape should be similar to figure 3. The ng/ml values can be read directly from the graph.



**Figure 3.** Typical standard curve for 2.5 hour incubation.

An alternative way of representing the data would be to simply plot Absorbance<sub>405</sub> values against MMP-14 standard concentrations. This type of plot would purely show the overall enzyme activity but not as a function of time.

## 9. Additional information

### 9.1. Specificity

The assay recognizes both the pro and active forms of MMP-14. Other MMPs have been assayed for cross reactivity (Table 3)

**Table 3.** Cross-reactivity

Compound	% 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*

\*Pro MMP-8 and Pro MMP-13 were added to cell cultures before extraction. All other cross-reactants were tested using purified MMP's, diluted in buffer, added to the assay as unknowns. They were APMA activated prior to dilution.

### 9.2. Reproducibility

#### Within assay precision

The within assay precision for duplicate determinations was calculated by measuring controls in the assay.

**Table 4** Within-assay precision (mean values as ng/ml)

Control	Mean±SD	%CV	n
L	3.19 ± 0.19	6.0	8
M	6.74 ± 0.24	3.6	8
H	17.4 ± 0.81	4.7	8

### Between-assay precision

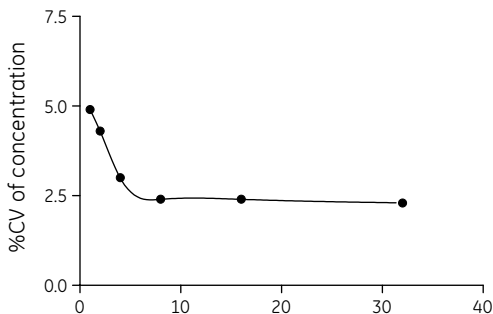
The between assay precision was assessed by repeat measurement of the same sample in successive assays. The results are shown below.

**Table 5.** Between-assay precision (mean values as ng/ml)

Control	Mean±SD	%CV	n
L	3.9 ± 0.71	18	9
M	6.05 ± 0.60	9.4	9
H	19.0 ± 2.9	15	9

### Precision profile

A precision profile was generated from the grand mean %CV of concentration for each standard point for 8 assays run in triplicate using one batch of in-house trial reagents. The results are shown in figure 4 below.



**Figure 4.** Precision dose profile

### 9.3. Sensitivity

The sensitivity, defined as two standard deviations above the mean optical density of 16 zero standard replicates, was determined for both ranges. The corresponding concentration was calculated from a standard curve. The mean zero and standard values were then used to calculate the sensitivity. This was determined as 0.7 ng/ml for the 1–32 ng/ml assay and 0.2 ng/ml for the more sensitive assay.

### 9.4. Recovery

The recovery of MMP-14 standard in tissue extract diluted 1:10 with extraction buffer is shown.

<b>Sample</b>	<b>Added conc (ng/ml)</b>	<b>Measured (ng/ml)</b>	<b>Expected (ng/ml)</b>	<b>Recovery (%)</b>
Tissue	0	0.6	-	-
extract	4	2.8	4.6	56.3
diluted 1 in 10	16	9.6	16.6	56.5
	32	15.8	32.6	47.5

## 9.5. Troubleshooting guide

<b>Problems</b>	<b>Checks</b>
<b>1. Low optical densities</b>	<ol style="list-style-type: none"><li>1. Check reader wavelength.</li><li>2. Check incubation time and temperature.</li><li>3. Check APMA activation procedure.</li><li>4. Reagents not equilibrated to correct temperature before use.</li><li>5. Check preparation of reagents.</li><li>6. Check kit reagents for improper storage.</li><li>7. Samples may contain high levels of the natural MMP inhibitors, TIMPs.</li></ol>
<b>2. High optical densities/high zero standard values</b>	<ol style="list-style-type: none"><li>1. Ensure that every well is completely filled and emptied at every wash step.</li><li>2. Ensure that automatic washers are functioning correctly.</li><li>3. Blot plates on tissue paper after washing.</li><li>4. Check incubation times and temperatures.</li><li>5. Check preparation of reagents.</li><li>6. Check time of preparation of detection reagents before use.</li></ol>
<b>3. Flat curves/poor reproducibility</b>	<ol style="list-style-type: none"><li>1. Check pipette calibration.</li><li>2. Check preparation of working standards.</li><li>3. Check that correct reagent volumes were added.</li><li>4. Check kit reagents for improper storage.</li></ol>

Problems	Checks
3. Flat curves/poor reproducibility <i>continued</i>	5. Check preparation of reagents. 6. Ensure that troughs used with multichannel pipettes are <b>separate</b> and <b>dedicated</b> to individual components.

## 9.6. Background and references

Matrix metalloproteinases (MMPs) are a family of  $Zn^{2+}$  endopeptidases that possess the ability to break down extracellular matrix macromolecules associated with tissue destruction in various pathological conditions(1). Their activity is not only regulated at the gene expression level but is strictly regulated by inhibitors, including an MMP-specific family called tissue inhibitors of metalloproteinases (TIMPs)(2). MMP expression is known to be controlled by pro- and anti-inflammatory cytokines and growth factors.

Overexpression and activation of MMPs or an imbalance of active MMPs and TIMPs, has been linked with a number of specific disease states associated with the breakdown and remodelling of the extracellular matrix, such as rheumatoid arthritis, periodontal disease, tumour invasion and metastasis, and vascular processes such as atherosclerosis, angiogenesis and aneurysms (3–8).

The MMPs can be grouped according to their domain structure into collagenases, gelatinases, stromelysins, matrilysin and membrane type MMPs (MT-MMPs) (1,3). MMP-8 (collagenase 2, neutrophil collagenase, EC3.4.24.34) has substrate specificity for native collagens including types I, II and III (9).

MMPs play a central role in pathological processes such as tissue destruction, fibrotic diseases and weakening of the matrix, including cancer invasion and metastasis.

MT1-MMP (MMP-14) is the first cloned and isolated membrane-type MMP. It contains a C-terminal proximal transmembrane domain and a short cytoplasmic tail in addition to domains shared with other MMPs. MT1-MMP is able to activate proMMP-2 and proMMP-13. TIMP-2 is thought to be involved in the binding of pro-MMP-2 to MT1-MMP (for review see refs. 10, 11) MT1-MMP has been detected in carcinomas of lung, cervix, stomach, colon and brain, developing embryos, placenta and cartilage (mostly based on immunohistochemistry and mRNA determination). The levels of MMP-14 correlate well with the activation of proMMP-2, supporting the idea that MMP-14 is an activator of proMMP-2. Tumor cells transfected with MMP-14 led to higher activation of MMP-2 and higher invasion of Matrigel. In addition to activation of MMP-2 and MMP-13, MT1-MMP is able to directly degrade ECM proteins such as interstitial collagens, fibronectin, vitronectin and laminin.

It is generally accepted that in most normal and neoplastic cells MT1-MMP is expressed as an active enzyme. The MT1-MMP propeptide has a furin recognition sequence (RRKR) at the C-terminus of the propeptide. This sequence is cleaved intracellularly resulting in the expression of active MT1-MMP at the cell membrane. It is suggested that human cells secrete soluble MT1-MMP and that MT1-MMP is released from the membrane by proteolytic cleavage.

We have developed an assay system, based on the QuickZyme™ technology, which enables the measurement of MMP-14 activity. This assay is specific and quantitative and can be applied to determine the levels of active MMP-14 in tissue extracts and tissue culture supernatants.

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