GE Healthcare

Amersham
Matrix Metalloproteinase-1, (MMP-1), Biotrak
Activity Assay System
96 wells
Product Booklet
Code: RPN2629
9.2.2. Typical assay data for 0.1–1.56 ng/ml standard range

10. Additional information
   10.1. Specificity
   10.2. Reproducibility
   10.3. Sensitivity
   10.4. Parallelism
   10.5. Recovery
   10.6. Expected values
   10.7. Troubleshooting guide
   10.8. Background and references
   10.9. Related products
1. Legal

GE and GE monogram are trademarks of General Electric Company.
Amersham and Biotok are trademarks of GE Healthcare companies.
Brij and Tween are trademarks of ICI Americas Inc.
S-2444 is a trademark of Chromogenix AB.
QuickZyme is a trademark of TNO Prevention and Health.
QuickZyme MMP-1 Activity Assay is based on technology developed in collaboration with TNO Prevention and Health, Gaubius Laboratory, PO Box 2215, 2301CE Leiden, The Netherlands.
© 2006 General Electric Company – All rights reserved.
GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specification and features shown herein, or discontinue the product described at any time without notice or obligation.
Contact your GE Healthcare representative for the most current information and a copy of the terms and conditions.
http://www.gehealthcare.com/lifesciences
GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK
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 material safety data sheet(s) and/or safety statement(s) for specific advice.

Note that the protocol requires the use of dimethylsulphoxide.

Warning: Dimethylsulphoxide is harmful and an irritant. Please follow the manufacturer’s safety data sheet relating to the safe handling and use of this material.

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 date of despatch.
3. Components of the assay systems

**Microplate**
The plate contains 12 x 8 well strips coated with anti-MMP-1, ready for use.

**Assay 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 µM zinc chloride and 0.01% (v/v) BRIJ™ 35.

**Standard**
Tube contains 100 ng of lyophilized human pro MMP-1. On reconstitution with 1 ml of buffer this gives a concentration of 100 ng/ml pro MMP-1 in 50 mM Tris-HCl buffer pH 7.6 containing 1.5 mM sodium chloride, 0.5 mM calcium chloride, 1 µM zinc chloride and 0.01% (v/v) BRIJ 35.

**p-Aminophenylmercuric acetate (APMA)**
Bottle contains 352 mg of p-aminophenylmercuric acetate (APMA) in a powder form.

**Detection enzyme**
Tube contains 100 µl 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 µM zinc chloride 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 µM zinc chloride 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% (v/v) Tween™ 20.
4. Additional materials and equipment required

The following materials and equipment are required but not supplied:

- Pipettes or pipetting equipment with disposable polypropylene tips (5 µl, 50 µl, 100 µl, 500 µl, 1 ml and 5 ml)
- Disposable polypropylene test tubes
- Glass measuring cylinders (100 ml and 500 ml)
- Distilled or deionized water
- Microplate shaker
- Refrigerator at 2–8°C
- Dimethylsulphoxide (DMSO), as supplied for tissue culture
- Microplate incubator at 37°C
- Spectrophotometric plate reader capable of measuring 405 nm
- Automatic plate washer or wash bottle
5. Description

- Specific for MMP-1
- Non-radioactive
- Microplate based assay
- Precise and accurate measurement
- Applicable to complex biological samples
- Ready to use detection enzyme and substrate
- Greater sensitivity than ELISA

The Biotrak™ MMP-1 activity assay system from GE Healthcare provides a simple, specific and precise quantitative determination of active or pro MMP-1 in tissue culture supernatants, serum, plasma, urine, synovial fluid and tissue homogenates.

Active MMP-1 may be measured in the range 3.13 to 50 ng/ml and the sensitivity of the assay is 2.2 ng/ml. A longer incubation protocol enables an assay range of 0.1 to 1.56 ng/ml with a sensitivity of 100 pg/ml, should higher sensitivity be required.

The assay uses the pro form of a detection enzyme that can be activated by captured active MMP-1, 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). Standards and samples are incubated in microplate wells precoated with anti-MMP-1 antibody. Any MMP-1 present will be bound to the wells, other components of the sample being removed by washing and aspiration. Either the endogenous levels of free active MMP-1 or the total levels of MMP-1 in a sample can be detected. In order to measure the total MMP-1 content, any bound MMP-1 in its pro form is activated using p-aminophenylmercuric acetate (APMA). The standard is pro MMP-1 which is activated in parallel for both types
of sample. Active MMP-1 is detected without APMA treatment. The resultant colour is read at 405 nm in a microplate spectrophotometer. The concentration of active MMP-1 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 42 samples in duplicate.

The Biotrak MMP-1 activity assay system has been specifically designed for research purposes.

Figure 1. Protocol for MMP-1 activity assay

**Note:** If total (pro and active) levels of MMP-1 are to be measured, APMA is added to the assay along with the detection reagent. Assay buffer is added instead of APMA if only endogenous levels of active MMP-1 are to be measured.
6. Critical parameters

The following points are critical:

1. The assay buffer concentrate, anti-MMP-1 coated microplate and wash buffer should be allowed to equilibrate to 20–27°C before preparation.

2. 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.

3. Use only coated wells from the same reagent batch for each assay.

4. The incubation temperatures are critical. Sample capture should be performed at 2–8°C, but all other incubations are performed at 37°C.

5. Preparation of working standards and addition of standards to microplate should be performed using polypropylene tips.

6. Once thawed out, samples, standards, detection enzyme and substrate should be kept at 2–8°C, prior to performing the assay.

7. Incubation times must be carried out exactly. If more than one is being assayed, each plate must be timed individually.

8. A separate standard curve must be run on each plate.

9. Mix samples and all reagents thoroughly before use.

10. Avoid excessive foaming of reagents.

11. Avoid handling the tops of wells both before and after filling.

12. Keep the wells covered with lids except when adding reagents and reading.

13. Standards and samples should be assayed in duplicate.

14. The total dispensing time for each plate should not exceed 20 minutes.
7. Specimen collection and sample preparation

The Biotrak MMP-1 activity assay system from GE Healthcare has been tested with several types of sample for which representative procedures are described for guidance. It remains the responsibility of the investigator to validate the chosen procedure.

7.1. Serum
1. Allow samples to coagulate and centrifuge.
2. Remove serum and store at -15°C to -30°C.
3. Avoid freeze thaw cycles.
4. Before assay, it is recommended to dilute serum samples 1:20 (20 fold dilution) or more, depending on the MMP-1 concentration in the sample. Assay buffer is provided for this.

7.2. Plasma
1. Collect plasma using heparin or citrate as an anticoagulant.
2. Centrifuge and remove plasma and store at -15°C to -30°C.
3. Avoid freeze thaw cycles.
4. Before assay, it is recommended to dilute heparin plasma samples 1:5 (5 fold dilution) or more, citrate 1:20 (20 fold dilution) or more, depending on the MMP-1 concentration in the samples. Assay buffer is provided for this.

Users should be aware that certain blood collection methods may alter the levels of MMP-1 measured in the assay (12). It is therefore recommended that heparin plasma is the preferred sample type.
7.3. Synovial fluid and urine
1. If necessary, centrifuge to remove any particulate material and store at -15°C to -30°C.
2. Before assay, it may be necessary to dilute synovial fluid samples before use if the samples are viscous. Urine should be diluted 1:1. Assay buffer is provided for this.

7.4. Cell culture supernatant
1. Ensure cells are cultured in serum-free media. If necessary, centrifuge to remove any particulate material and store at -15°C to -30°C.
2. It may be necessary to dilute the samples if levels of MMP-1 are high. Assay buffer is provided for this.

7.5. Tissue samples
Users are advised to carefully validate any tissue extraction procedure employed. The following method is described for the preparation of tissue samples from breast tumours (13). It is suggested for guidance only, as its use has not been validated with this assay or with other tissues.
1. Homogenize tumours in 50 mM Tris-HCl buffer pH 7.4 containing 1 mM monothioglycerol.
2. Centrifuge at 2000 x g for 10 minutes.
3. Assay the supernatant for MMP-1
8. Assay procedure

8.1. Reagent preparation

The assay buffer concentrate, anti-MMP-1 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

Assay 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.

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

Standard
1. Add 1 ml of assay buffer to the vial and replace the cap.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Store on ice until required.

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.
3. Store at room temperature in a closed vessel until required for use.

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

Day 2
p-Aminophenylmercuric acetate (APMA)
1. Add 1 ml of fresh dimethylsulphoxide (DMSO) to the bottle and replace the stopper.
2. Vortex the bottle until all of the powder has gone into solution. This is the concentrated APMA solution (1 M).
3. Add 5 µl of the concentrated solution (1 M) from 2, to a vial containing 20 ml of room temperature equilibrated assay buffer and vortex to mix.
4. Add 1 ml of the solution from 3, to a vial containing 9 ml of room temperature equilibrated assay buffer and vortex to mix. This is the ready to use APMA solution (0.025 mM).

The concentrated APMA solution should be stored at room temperature. This concentrated APMA solution can be re-used for a period of 4 weeks. The ready to use APMA solution should be made up fresh for each application and discarded after use.

Detection enzyme
1. Allow the tube containing the detection enzyme to thaw for 10 minutes 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 only be prepared immediately prior to addition to the wells.
1. For every 9 wells take 10 µl of the detection enzyme concentrate and add to 500 µl of the reconstituted substrate.
2. If using the whole plate add 100 µl of the detection enzyme concentrate to the reconstituted substrate.
3. Mix gently but thoroughly.
4. 50 µl of detection reagent is added to each well during the assay protocol (see pages 16-17).

Once reconstituted the assay 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 3.13–50 ng/ml (higher MMP-1 levels)
(See pages 18-19 for an assay range 0.1–1.56 ng/ml [lower MMP-1 levels])

8.2.1. Preparation of working standards
Note: It is important to use a clean polypropylene pipette tip for each dilution.
1. Label 5 polypropylene tubes for 3.13, 6.25, 12.5, 25 and 50 ng/ml.
2. Pipette 500 µl of assay buffer into each tube.
3. Pipette 500 µl of the stock standard (100 ng/ml) into the 50 ng/ml tube.
4. Vortex mix.
5. Pipette 500 µl from the 50 ng/ml tube into the 25 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 5 standard levels of pro MMP-1 ranging from 3.13 to 50 ng/ml.

*Note: Stock solution at 100 ng/ml is not part of the standard curve. This may be stored at -15°C to -30°C if only running a part plate.*

Working standards should be freshly prepared before each assay and not reused.

8.2.2. Assay protocol
1. Prepare the reagents as described in ‘reagent preparation’.
2. Prepare the working standards as described in previous section.
3. Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required.
4. Pipette 100 µl of assay buffer into the zero standard wells.
5. Pipette 100 µl of each standard into the appropriate wells, using a clean polypropylene pipette tip for each standard.
6. Pipette 100 µl of unknown sample into the appropriate wells.
7. Cover the plate with the lid provided and incubate at 2–8°C overnight.
8. Aspirate and wash all wells 4 times with wash buffer ensuring that the wells are completely filled and emptied at each wash.
9. Blot the plate on tissue paper ensuring any residual volume is removed during the blotting procedure.
10. Prepare the detection reagent as described in the reagent preparation section.
11. Briefly vortex the detection reagent to ensure thorough mixing.
12. Pipette 50 µl of ready to use APMA solution into wells containing standards, and into those wells containing samples where total (pro and active) MMP-1 activity is to be measured.

13. Pipette 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-1 are to be measured.

14. Pipette 50 µl of the detection reagent into all wells.

15. Shake the plate for 20 seconds.

16. Read the plate at 405 nm to obtain a t0 value.

17. Cover the plate with the lid provided and incubate at 37°C for 90 minutes.

18. Shake the plate for 20 seconds.

19. Read the plate at 405 nm.
8.3. Assay range 0.1–1.56 ng/ml (for lower MMP-1 levels)

8.3.1. Preparation of working standards

**Note:** It is important to use a clean polypropylene pipette tip for each dilution.

1. Label 10 polypropylene tubes for 0.1, 0.2, 0.4, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ng/ml.
2. Pipette 500 µl of assay buffer into each tube.
3. Pipette 500 µl of the stock standard (100 ng/ml) into the 50 ng/ml tube.
4. Vortex mix.
5. Pipette 500 µl from the 50 ng/ml tube into the 25 ng/ml tube.
6. Vortex mix.
7. Repeat this doubling dilution successively with the remaining tubes.
8. Discard the 3.13, 6.25, 12.5, 25 and 50 ng/ml tubes, leaving 5 standard levels of MMP-1 ranging from 0.1 to 1.56 ng/ml.
9. 100 µl aliquots from each remaining serial dilution will give rise to 5 standard levels of pro MMP-1 ranging from 0.1 to 1.56 ng/ml.

**Note:** Stock solution at 100 ng/ml is not part of the standard curve. This may be stored at -15°C to -30°C if only running a part plate.

Working standards should be freshly prepared before each assay and not reused.

8.3.2. Assay protocol

1. Prepare the reagents as described in ‘reagent preparation’.
2. Prepare the working standards as described in previous section.
3. Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required.
4. Pipette 100 µl of assay buffer into the zero standard wells.
5. Pipette 100 µl of each standard into the appropriate wells, using a clean polypropylene pipette tip for each standard.
6. Pipette 100 µl of unknown sample into the appropriate wells.
7. Cover the plate with the lid provided and incubate at 2–8°C overnight.
8. Aspirate and wash all wells 4 times with wash buffer ensuring that the wells are completely filled and emptied at each wash.
9. Blot the plate on tissue paper ensuring any residual volume is removed during the blotting procedure.
10. Prepare the detection reagent as described in the reagent preparation section.
11. Briefly vortex the detection reagent to ensure thorough mixing.
12. Pipette 50 µl of ready to use APMA solution into wells containing standards, and into those wells containing samples where total (pro and active) MMP-1 activity is to be measured.
13. Pipette 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-1 are to be measured.
14. Pipette 50 µl of the detection reagent into all wells.
15. Shake the plate for 20 seconds.
16. Read the plate at 405 nm to obtain a t₀ value.
17. Cover the plate with the lid provided and incubate at 37°C for 4 hours.
18. Shake the plate for 20 seconds.
19. Read the plate at 405 nm.
Note: The detection incubation can be continued for longer if required. Users who find their OD₄₅₀ values to be lower than those quoted in the typical data section can do this to increase their optical densities accordingly.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>O</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>B</td>
<td>0.13</td>
<td>0.13</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C</td>
<td>0.25</td>
<td>0.25</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>D</td>
<td>1.25</td>
<td>1.25</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>E</td>
<td>5.0</td>
<td>5.0</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>F</td>
<td>12.5</td>
<td>12.5</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>G</td>
<td>25</td>
<td>25</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>H</td>
<td>50</td>
<td>50</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Figure 2. Recommended positioning of standard and sample (S) wells. For lower endogenous MMP-1 levels a curve range of 0.1–1.56 ng/ml is recommended.
9. Data processing

9.1. Calculation of results
The calculation is illustrated using representative data.

As MMP-1 activity is directly proportional to the generation of colour 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$_{405}$ values 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 50 ng/ml standard at a 1.5 hour detection incubation period. Rate of change of MMP activity should be expressed as:

$$\frac{(\text{Abs}_{t=1.5} - \text{Abs}_{t=0}) \times 1000}{h^2}$$

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

$$(\delta \text{Abs}_{t=1.5}/h^2) \times 1000$$

For a 1.5 hour detection incubation:
Mean absorbance$_{405}$ for 50 ng/ml standard = 0.694
Mean absorbance$_{405}$ for 0 ng/ml standard (blank) = 0.074
\[ \delta \text{Absorbance}_{405} = 0.620 \]

For a 1.5 hour incubation period:

\[ \delta \text{Absorbance}_{405}/h^2 = \frac{0.620}{2.25} = 0.27556 \]

\[ \delta \text{Absorbance}_{405}/h^2 \times 1000 = 0.27556 \times 1000 = 275.56 \]

9.2. Typical assay data

9.2.1. Typical assay data for 3.13–50 ng/ml standard range

The assay data for the standard curve (3.13–50 ng/ml) should be similar to that shown in table 1.

**Table 1. Typical assay data for a standard curve (90 minute incubation)**

<table>
<thead>
<tr>
<th>MMP-1 (ng/ml)</th>
<th>Absorbance\text{\textsubscript{405}}</th>
<th>Mean absorbance\text{\textsubscript{405}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.689</td>
<td>0.694</td>
</tr>
<tr>
<td></td>
<td>0.698</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.466</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td>0.427</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>0.266</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td>0.270</td>
<td></td>
</tr>
<tr>
<td>6.25</td>
<td>0.169</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>3.13</td>
<td>0.122</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.074</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>0.074</td>
<td></td>
</tr>
</tbody>
</table>

For a 1.5 hour incubation period the raw data in table 1 would therefore be plotted as shown in table 2.
Table 2. Raw data expressed as $\Delta \text{Absorbance}_{405}/h^2 \times 1000$

<table>
<thead>
<tr>
<th>MMP-1 (ng/ml)</th>
<th>Mean $\Delta \text{Absorbance}_{405}/h^2 \times 1000$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>275.56</td>
</tr>
<tr>
<td>25</td>
<td>165.58</td>
</tr>
<tr>
<td>12.5</td>
<td>86.22</td>
</tr>
<tr>
<td>6.25</td>
<td>42.67</td>
</tr>
<tr>
<td>3.13</td>
<td>21.78</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A standard curve is generated by plotting $\Delta \text{Abs}_{405,1.5}/h^2 \times 1000$ (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 90 minute incubation

An alternative way of representing the data would be to simply plot Absorbance$_{405}$ values against MMP-1 standard concentrations. This type of plot would purely show the overall enzyme activity but not as a function of time.
9.2.2. Typical assay data for 0.1–1.56 ng/ml standard range

Table 3. Typical assay data for a standard curve (4 hour incubation)

<table>
<thead>
<tr>
<th>MMP-1 (ng/ml)</th>
<th>Absorbance$_{405}$</th>
<th>Mean absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.56</td>
<td>0.323</td>
<td>0.331</td>
</tr>
<tr>
<td></td>
<td>0.338</td>
<td></td>
</tr>
<tr>
<td>0.78</td>
<td>0.244</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>0.185</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>0.156</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>0.156</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>0.146</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.131</td>
<td>0.131</td>
</tr>
</tbody>
</table>

Figure 4. Typical standard curve for 4 hour incubation
10. Additional information

10.1. Specificity
The assay recognizes pro and active forms of MMP-1. It does not cross-react with other MMPs or TIMPs (table 4).

Table 4. Cross-reactivity

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>pro MMP-1</td>
<td>100</td>
</tr>
<tr>
<td>Active MMP-1</td>
<td>100</td>
</tr>
<tr>
<td>Active MMP-1/TIMP-1 complex</td>
<td>12.5</td>
</tr>
<tr>
<td>MMP-2</td>
<td>&lt;0.620</td>
</tr>
<tr>
<td>MMP-3</td>
<td>&lt;0.645</td>
</tr>
<tr>
<td>MMP-8</td>
<td>&lt;0.310</td>
</tr>
<tr>
<td>MMP-9</td>
<td>1.000</td>
</tr>
<tr>
<td>MMP-13</td>
<td>0.063</td>
</tr>
<tr>
<td>MMP-14</td>
<td>0.500</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>&lt;1.29</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>&lt;1.29</td>
</tr>
</tbody>
</table>

This data was obtained using pre-activated MMP-1 that was captured and washed in the assay, without being further APMA activated.

10.2. Reproducibility

Within-assay precision
The within-assay precision for duplicate determinations was calculated by measuring controls in the assay. The results are shown in table 5.
Table 5. Within-assay precision (mean value as ng/ml)

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean ± SD</th>
<th>%CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>5.68 ± 0.52</td>
<td>9.2</td>
<td>12</td>
</tr>
<tr>
<td>M</td>
<td>11.91 ± 0.78</td>
<td>6.6</td>
<td>12</td>
</tr>
<tr>
<td>H</td>
<td>25.14 ± 1.48</td>
<td>5.9</td>
<td>12</td>
</tr>
</tbody>
</table>

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

Table 6. Between-assay precision (mean values as ng/ml)

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean ± SD</th>
<th>%CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>6.70 ± 1.58</td>
<td>23.6</td>
<td>12</td>
</tr>
<tr>
<td>M</td>
<td>14.51 ± 2.09</td>
<td>14.4</td>
<td>12</td>
</tr>
<tr>
<td>H</td>
<td>26.63 ± 4.03</td>
<td>15.1</td>
<td>12</td>
</tr>
</tbody>
</table>

Precision profile
A precision profile was generated from the grand mean %CV of concentration for each standard point for 12 assays run in triplicate using one batch of in-house trial reagents. The results are shown in figure 5.
10.3. Sensitivity
The sensitivity, defined as two standard deviations above the mean optical density of 10 zero standard replicates, was determined for the 3.13 to 50 ng/ml range. 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 2.2 ng/ml for a 1.5 hour incubation period. A detection sensitivity of 100 pg/ml has however been calculated for the increased sensitivity protocol, using a 0.1 to 1.56 ng/ml standard range.

10.4. Parallelism
Serum and plasma samples were assayed after dilution with assay buffer to assess linearity of the assay. MCF-7 cells were stimulated with PMA/TNFα and the conditioned media used to assess linearity after dilution in serum-free media.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>%Observed Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture</td>
<td>1:1</td>
<td>36.58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>medium</td>
<td>1:2</td>
<td>20.64</td>
<td>18.29</td>
<td>112.0</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>8.57</td>
<td>4.57</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>3.54</td>
<td>4.57</td>
<td>75.0</td>
</tr>
<tr>
<td>Heparin</td>
<td>1:5</td>
<td>0.82</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>plasma</td>
<td>1:10</td>
<td>0.38</td>
<td>0.41</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>0.15</td>
<td>0.20</td>
<td>75.0</td>
</tr>
<tr>
<td>Citrate</td>
<td>1:10</td>
<td>1.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>plasma</td>
<td>1:20</td>
<td>0.62</td>
<td>0.68</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>0.39</td>
<td>0.34</td>
<td>114.7</td>
</tr>
<tr>
<td>Serum</td>
<td>1:20</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>0.43</td>
<td>0.42</td>
<td>97.7</td>
</tr>
</tbody>
</table>

EDTA plasma sample measurement is not recommended with this product. EDTA is able to chelate Zn$^{2+}$ from the MMP-1 and therefore has adverse effects on its activity.

10.5. Recovery

Serum and plasma samples were diluted 1:10 with assay buffer and spiked with known concentrations of MMP-1. Serum-free culture medium was also spiked with a range of MMP-1 concentrations. The recovery of MMP-1 activity was calculated after measurement using the MMP-1 assay.
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Added conc. (ng/ml)</th>
<th>Measured (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medium</td>
<td>0</td>
<td>4.87</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>7.61</td>
<td>10.06</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>13.90</td>
<td>19.53</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32.0</td>
<td>28.49</td>
<td>112.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>63.7</td>
<td>59.25</td>
<td>107.5</td>
</tr>
<tr>
<td>Citrate</td>
<td>0</td>
<td>0.98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>plasma</td>
<td>0.4</td>
<td>0.74</td>
<td>1.38</td>
<td>53.6</td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>1.31</td>
<td>1.76</td>
<td>74.4</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.37</td>
<td>2.54</td>
<td>53.9</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>1.87</td>
<td>4.11</td>
<td>45.5</td>
</tr>
<tr>
<td>Heparin</td>
<td>0</td>
<td>1.34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>plasma</td>
<td>0.4</td>
<td>1.52</td>
<td>1.74</td>
<td>87.4</td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>1.89</td>
<td>2.12</td>
<td>89.2</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>2.47</td>
<td>2.90</td>
<td>85.2</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>3.15</td>
<td>4.47</td>
<td>70.5</td>
</tr>
<tr>
<td>Serum</td>
<td>0</td>
<td>1.18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>1.40</td>
<td>1.58</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>1.24</td>
<td>1.96</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.52</td>
<td>2.74</td>
<td>55.5</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>1.42</td>
<td>4.31</td>
<td>32.9</td>
</tr>
</tbody>
</table>
10.6. Expected values

Normal serum and plasma samples were evaluated in the assay.

Table 9. Levels of MMP-1 in human serum and plasma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>Range (ng/ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin plasma</td>
<td>17.9</td>
<td>5.3–50</td>
<td>4</td>
</tr>
<tr>
<td>Citrate plasma</td>
<td>11.8</td>
<td>8.6–17.5</td>
<td>4</td>
</tr>
<tr>
<td>Serum</td>
<td>19.9</td>
<td>16.0–23.8</td>
<td>4</td>
</tr>
</tbody>
</table>
### 10.7. Troubleshooting guide

<table>
<thead>
<tr>
<th>Problems</th>
<th>Checks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Low optical densities</strong></td>
<td>1. Check reader wavelength.</td>
</tr>
<tr>
<td></td>
<td>2. Check incubation time and temperature.</td>
</tr>
<tr>
<td></td>
<td>3. Check APMA activation procedure.</td>
</tr>
<tr>
<td></td>
<td>4. Reagents not equilibrated to correct temperature before use.</td>
</tr>
<tr>
<td></td>
<td>5. Check preparation of reagents</td>
</tr>
<tr>
<td></td>
<td>6. Check kit reagents for improper storage.</td>
</tr>
<tr>
<td></td>
<td>7. Samples may contain high levels of the natural MMP inhibitors, TIMPs.</td>
</tr>
<tr>
<td><strong>2. High optical densities/ or high zero standard values</strong></td>
<td>1. Ensure that every well is completely filled and emptied at every wash step.</td>
</tr>
<tr>
<td></td>
<td>2. Ensure that automatic washers are functioning correctly.</td>
</tr>
<tr>
<td></td>
<td>3. Blot plates on tissue paper after washing.</td>
</tr>
<tr>
<td></td>
<td>4. Check incubation time and temperature.</td>
</tr>
<tr>
<td></td>
<td>5. Check preparation of reagents</td>
</tr>
<tr>
<td></td>
<td>6. Check time of preparation of detection reagents before use.</td>
</tr>
<tr>
<td></td>
<td>7. Check that correct reagent volumes were added.</td>
</tr>
</tbody>
</table>
Problems | Checks
---|---
3. Flat curves / poor reproducibility | 1. Check pipette calibration.  
2. Check preparation of working standards.  
3. Check that the correct reagent volumes were added.  
4. Check kit reagents for improper storage.  
5. Check preparation of reagents.  
6. Ensure that troughs used with multichannel pipettes are separate and dedicated to individual components.  
7. Insufficient washing procedure.

10.8. 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. Over-expression 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 arthritis, cancer and tumour invasion and metastasis (1, 3).

The MMPs can be grouped according to their domain structure into collagenases, gelatinases, stromelysins, matrilysin and membrane type MMPs (MT-MMPs) (1, 4). MMP-1 (interstitial collagenase, EC 3. 4. 24. 7) can cleave collagen helices to yield characteristic one-quarter to three-quarter products (5). MMP-1 is expressed in
fibroblasts, macrophages, chondrocytes, and certain tumour cells, and is implicated in many disease states. It is secreted as a latent proenzyme of 52 kDa MW which is N-glycosylated to a minor form of 57 kDa. It can be activated in vitro by proteinases (e.g. trypsin and plasmin), mercurials (e.g. p-aminophenylmercuric acetate, APMA) and in vivo by plasmin and MMP-3 (stromelysin-1) (6). Active MMP-1 can form a complex with TIMP-1 in a 1:1 molar ratio. Although sequences are well conserved across species (>50% between enzymes), the collagenases MMP-1 and MMP-13 are most related, sharing 86% identity at the amino acid level. MMP-8 shares only 57% identity with MMP-1 (7).

There are several assays for MMP activity such as the use of short peptide substrates which, when cleaved by the MMP of interest, release a detectable marker. These however do not offer complete specificity, e.g. between MMP-1, MMP-8, MMP-13 and also lack sensitivity. Several immunoassays to MMP-1 have now been described (8–10).

We have developed an assay system (11) which enables the measurement of MMP-1 activity. The assay uses the QuickZyme™ detection enzyme, in its pro form, which can be activated by captured active MMP-1, or APMA activated pro MMP-1, 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 recognised by specific matrix metalloproteinases. MMP activated detection enzyme can then be measured using a specific chromogenic peptide substrate. This assay is specific and quantitative and can be applied to determine the levels of active MMP-1 in various samples, such as serum, plasma, cell culture medium, urine, synovial fluid and tissue homogenate.


10.9. Related products
The Biotrak range of MMP and related ELISA systems

- MMP-1, human RPN2610
- TIMP-1, human RPN2611
- MMP-3, human RPN2613
- MMP-3, rabbit RPN2615
- MMP-9, human RPN2614
- MMP-2, human RPN2617
- TIMP-2, human RPN2618
- MMP-8, human RPN2619
- MMP-7, human RPN2620
- MMP-13, human RPN2621
- MMP-2 activity assay RPN2631
- MMP-13 activity assay RPN2632
- MMP-9 activity assay RPN2634

Please contact your local GE Healthcare office for details.