

# DNA Yields from ZyGEM's *prepGEM*<sup>™</sup> & *forensicGEM*<sup>™</sup>



Mt Erebus, Antarctica... the source of *prepGEM*<sup>™</sup>.

## DNA Yields

### CONTENT

#### 1.1

#### ENZYME PROPERTIES

Stability data

Response to inhibitors

Comparative activity

#### 1.2

#### DNA YIELDS

Liquid blood

Buccal swabs

### 1.1 Enzyme Properties

#### Stability Data

*prepGEM* is stabilized by Ca<sup>2+</sup>.  
In presence of 5 mM CaCl<sub>2</sub>.

75°C no loss of activity  
after 7 days; t<sub>1/2</sub> = 1h

85°C t<sub>1/2</sub> = 60 min (10 mM  
CaCl<sub>2</sub> t<sub>1/2</sub> = 115 min)

95°C t<sub>1/2</sub> = 10min

However, Ca<sup>2+</sup> is not added to the storage buffer because this divalent ions are undesirable in the PCR. As a consequence, the proteinase as prepared by ZyGEM has a reduced stability but is still sufficiently robust for easy handling and transportation. In the storage buffer (with 20% Glycerol) there is only a 10% loss of activity after 100 days at room temperature. In the standard reaction buffer (without glycerol) *prepGEM*<sup>™</sup> loses only 20% activity after a year at room temperature (Figure 1).

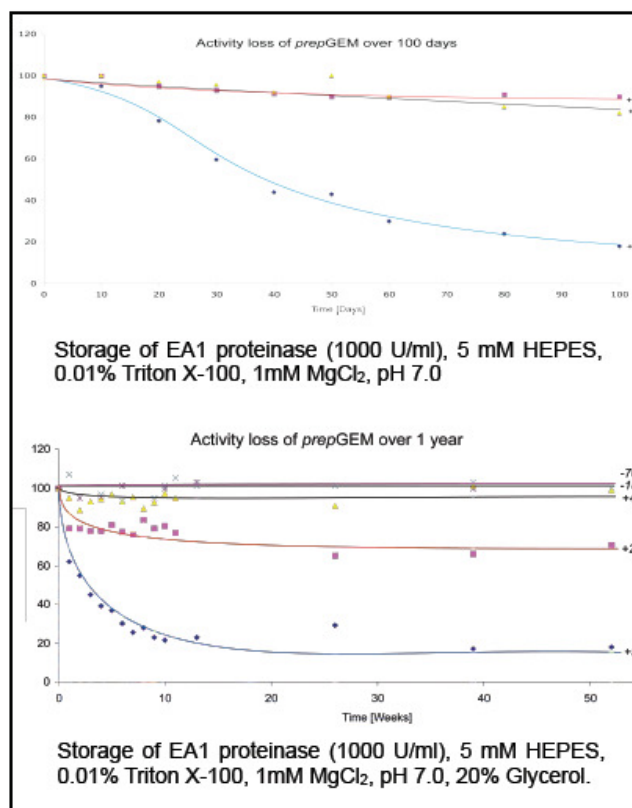
#### Stability Under Laboratory Abuse

Typically, tubes of enzymes are repeatedly

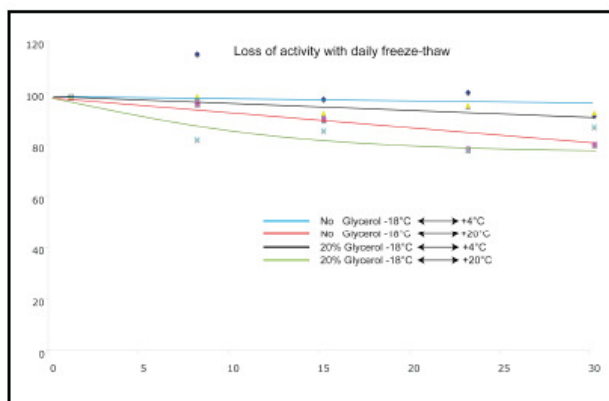
removed from storage at -20°C and transferred either to ice or to room temperature. Figure 2 shows the loss of activity of *prepGEM*<sup>™</sup> over 30 daily cycles of freeze-thaw. Negligible loss can be seen when the temperature shift is just above freezing point and a loss of approximately 15% is seen when the enzyme is transferred from the freezer to room temperature 30 times.

**Figure 1.**

Rate of activity loss of *prepGEM*<sup>™</sup> in storage buffer with & without Glycerol.



**Figure 2.**  
Rate of activity loss of *prepGEM*<sup>TM</sup> under a variety of freeze-thaw cycles.



### Response to Inhibitors

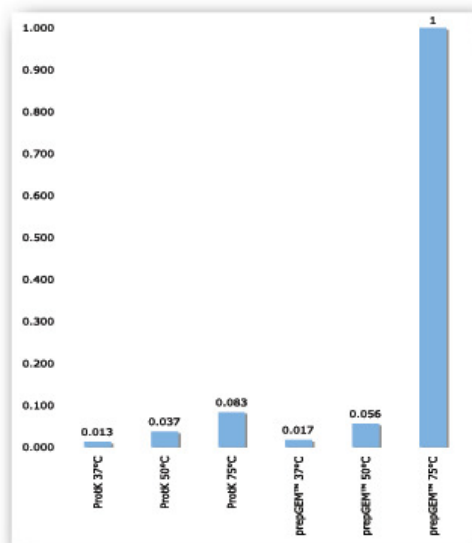
The following data were generated using Azocasein as substrate and 0.02% inhibitor.

Inhibitor	% Inhibited
Gramicidin S	29.60
Trypsin (soy bean)	9.05
Trypsin (egg)	5.97
Gramicidin	3.90
Trypsin (lima bean)	2.26
Bacitran	0.82
Benzamidine	0.00
Control	0.00

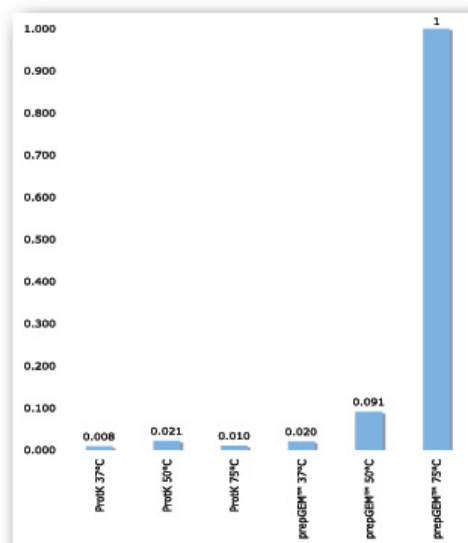
### Comparative specific activity with Proteinase K

**Figure 3.**  
Specific proteolytic rates of proteinase K and *prepGEM*<sup>TM</sup> at different temperatures. The substrate used was 0.2% BSA. Values are shown relative to the specific activity of *prepGEM*<sup>TM</sup> at 75°C.

#### 10 minute assay



#### 100 minute assay



## 1.2 DNA yields

### Liquid Blood

The following yields were calculated for *forensicGEM*<sup>TM</sup> using both a blot-based system, ABI Quantiblot® and a qPCR method, Quantifiler®.

Sample size = 2.5 µl Blood

Final Volume *forensicGEM*<sup>TM</sup> extraction = 100 µl

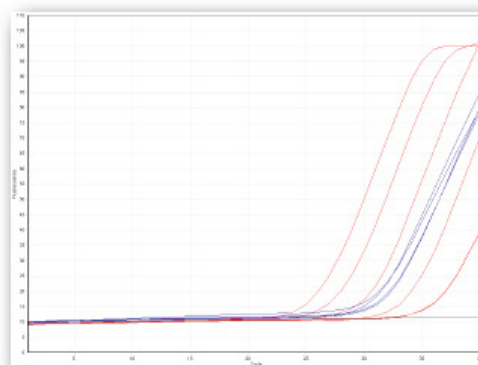
- QIAGEN report typical yields of 3 µg of DNA from 2 ml of blood (2.5 x 10<sup>5</sup> Leukocytes per ml) for their QIAamp columns. This equates to a yield of 1.5 ng DNA / µl of blood.
- Chemagen report yields of 60-120 µg of DNA from 3 ml of blood. This equates to a total yield of 20 – 40 ng DNA / µl of blood.

	Quantiblot	Quantifiler
<b>TOTAL YIELD:</b> ng per µl blood	25.2 ± 7.0	44.1 ± 22.8
<b>CONC.</b> in extract ng / µl	0.6 ± 0.2	0.82 ± 0.46

**Figure 4 a).**

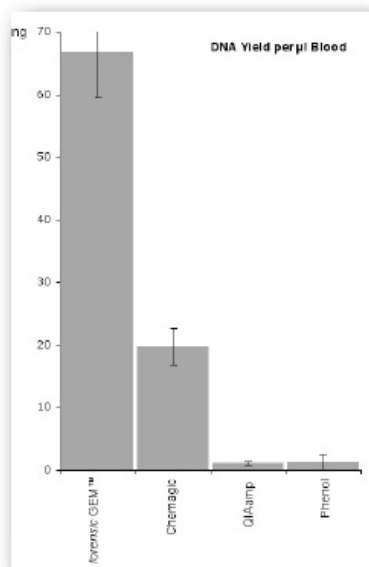
Blue: Examples of Quantifiler® traces from *forensicGEM*<sup>TM</sup> blood extracts. Samples from four individuals were used.

Red: standards generated using the manufacturer's DNA



**Figure 4 b).**

Yields from blood samples (four individuals) using *forensicGEM*<sup>TM</sup> and other extraction methods. Error bars are one standard deviation.



	Vol. of blood used	Final Vol. of extract
<i>forensicGEM</i> <sup>TM</sup>	2.5	100
Chemagic	50	100
QIAamp	5	50
Phenol	5	50

Note: Different blood samples were used to create this data and the quantification table above. Variation is typical between blood samples due to different titres of white blood cells.

**Notes:**

- The method for *forensicGEM*<sup>TM</sup> was as follows:
  - The following extraction mixture was made:
    - 2.5 µl of fresh blood
    - 97 µl of Buffer 3.
    - 1 µl of *forensicGEM*<sup>TM</sup>
  - Samples were incubated at 75 °C for 15 minutes and 95°C for 5 - 15 minutes.
  - The extracts were centrifuged at 13,000 x g for 5 minutes.
- The phenol method used 20 µl of blood treated with proteinase K in a 200 µl volume. Samples were extracted with phenol, phenol+chloroform+IAA (25:24:1) and chloroform. Samples were precipitated in ethanol and then resuspended on 50 µl of TE buffer.

All other methods were scaled variants of the manufacturer's instructions.

**Buccal Swabs**

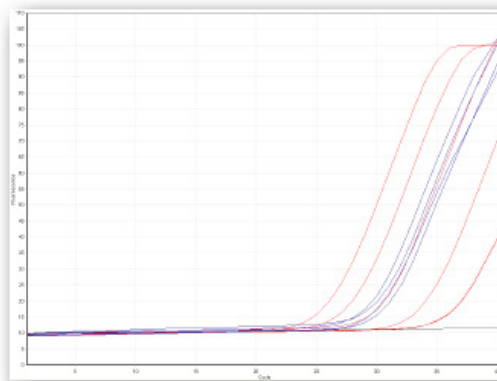
The following yields were calculated for *forensicGEM*<sup>TM</sup> using both a blot-based system, ABI Quantiblot® and a qPCR method, Quantifiler®.

	Quantiblot	Quantifiler
<b>TOTAL YIELD:</b>		
ng per µl ¼ swab	175 ± 101.5	235 ± 88.9
<b>CONC.</b>		
in extract ng /µl	1.8 ± 1.02	2.4 ± 0.89

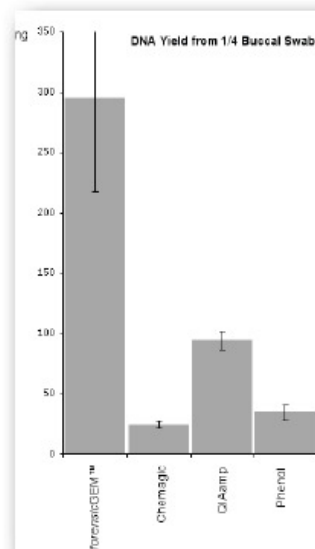
Sample size = ¼ of a swab  
Final Volume *forensicGEM*<sup>TM</sup> extraction = 100 µl

- On this basis a complete swab would be expected to yield ~ 940 ng of DNA.
- Large variation in yields is common with buccal swabs samples would be expected to vary from between 500 and 2000 ng. QIAGEN reports similar yields for the QIAamp 96 DNA Swab BioRobot Procedure.

**Figure 5a).**



**Blue:** Examples of Quantifiler® traces from *forensicGEM*<sup>TM</sup> buccal swab extracts. Four extractions are shown.  
**Red:** Standards generated using the manufacturer's DNA



**Figure 5b).**

Yields from buccal swabs (four individuals) using *forensicGEM*<sup>TM</sup> and other extraction methods. Error bars are one standard deviation

Preparation of Swab samples - Individual swabs were washed in 400 µl of buffer. This suspension was used the four extraction methods.

	Vol. of suspension used	Final Vol. of extract:
<i>forensicGEM</i> <sup>TM</sup>	100	100
Chemagic	100	100
QIAGEN	100	50
Phenol	100	50

Note: Different Buccal swabs were used to create this data and the quantification table above. Buccal swabs vary greatly from sample to sample.

**Notes:**

1. The method used for *forensicGEM™* was as follows:
  - a. The following extraction mixture was made:
    - i. 100 µl of swab suspension
    - ii. 2 µl of 50 x Buffer 3H.
    - iii. 1 µl of *forensicGEM™*
  - b. Samples were incubated at 75 °C for 15 minutes and 95°C for 5 - 15 minutes.

All other methods were scaled variants of the manufacturer's instructions.

---