

Supplemental data

Table 1.

<i>Step</i>	<i>Volume of fraction (ml)</i>	<i>Concentration (mg/ml)</i>	<i>Total amount (mg)</i>	<i>Activity (units)</i>	<i>Specific activity (units/mg protein)</i>	<i>Yield (%)</i>	<i>Purification factor fold</i>
<i>Cell lysate</i>	<i>100</i>	<i>13.5</i>	<i>1350</i>	<i>22.3</i>	<i>0.0165</i>	<i>100</i>	<i>1.0</i>
<i>Protamine sulfate</i>	<i>100</i>	<i>5.63</i>	<i>563</i>	<i>11.3</i>	<i>0.020</i>	<i>50.6</i>	<i>1.21</i>
<i>Ni-column</i>	<i>17.43</i>	<i>0.15</i>	<i>2.7</i>	<i>7.2</i>	<i>2.66</i>	<i>32.28</i>	<i>161.21</i>
<i>Phenyl sepharose</i>	<i>8.5</i>	<i>0.21</i>	<i>1.8</i>	<i>6.1</i>	<i>3.38</i>	<i>27.35</i>	<i>204.84</i>
<i>Q-Sepharose</i>	<i>2.3</i>	<i>0.69</i>	<i>1.6</i>	<i>5.8</i>	<i>3.625</i>	<i>26.00</i>	<i>219.69</i>

Table 1. Bacterial ceramidase was purified from two liters of culture as described in experimental procedures. One enzyme unit was defined as the amount capable of hydrolyzing 1 μ mol ceramide/ min at 37°C using D-erythro-C16 ceramide as a substrate at a concentration of 50 μ M in a 50 mM (pH 7.1) Tris- HCl buffer containing 1 mM CaCl₂, 0.1% (w/v) Triton X-100 final concentration, with a total volume of 100 μ l.

Table 2.

<i>Step</i>	<i>Volume of fraction (ml)</i>	<i>Concentration (mg/ml)</i>	<i>Total amount (mg)</i>	<i>Activity (units)</i>	<i>Specific activity (units/mg protein)</i>	<i>Yield (%)</i>	<i>Purification factor fold</i>
<i>Cell lysate</i>	<i>100</i>	<i>7.56</i>	<i>756</i>	<i>17.32</i>	<i>0.0229</i>	<i>100</i>	<i>1.0</i>
<i>Protamine sulfate</i>	<i>95</i>	<i>3.7</i>	<i>352</i>	<i>9.43</i>	<i>0.026</i>	<i>54.44</i>	<i>1.135</i>
<i>Ni-column</i>	<i>25</i>	<i>0.048</i>	<i>1.2</i>	<i>6.23</i>	<i>5.19</i>	<i>35.96</i>	<i>226.6</i>
<i>Phenyl sepharose</i>	<i>2</i>	<i>0.35</i>	<i>0.7</i>	<i>2.4</i>	<i>3.42</i>	<i>13.8</i>	<i>149.34</i>

Table 2. Sphingomyelinase D was purified from four liters of culture as described in experimental procedures. One enzyme unit was defined as the amount capable of hydrolyzing 1 μ mol sphingomyelin/ min at 37°C using D-erythro-C16-sphingomyelin as a substrate at a concentration of 50 μ M in a 10 mM (pH 7.4) HEPES buffer containing 1 mM CaCl₂, 5 mM KCl, 1 mM MgCl₂, with a total volume of 100 μ l.