PROFILING AND ANALYSIS OF STARCH SYNTHASE AT DIFFERENT TRUNK HEIGHTS OF SAGO PALM
(*Metroxylon sagu* ROTTB.)

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DECLARATION

I hereby declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institution of higher learning.

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ABSTRACT

Research on sago palm has been a main focus in Sarawak state as it has a great potential to boost the economy. Various studies have been performed on this starchy plant and one of it is the study on its starch biosynthesis pathway. Starch synthase was identified as one of the enzymes that play vital role in biosynthesis of starch. Therefore the profiling and characterization of starch synthase has been studied. Three palms were sampled from Bau district, specifically from Kampung Tanjong and Kampung Sidoh. Targeted part for studies on the palm wasting base height, middle height, and top height of the trunk. Initial studies were the optimization of specific extraction protocol for starch synthase from sago palm with the GBSS buffer was identified as the most suitable buffer. The best method for post extraction purification and concentration was determined as the cold acetone precipitation method. Then total starch content in 1 g/mL of sample and total protein concentration was measured and undergoes ANOVA, with the statistical analysis showed no significant difference (p < 0.05) of total starch content in 1 g/mL of sample and total protein concentration among each palms. However ANOVA on the data for starch content between the inner part and outer part of the trunk showed the outer part contained more starch than the inner part. Presence of Starch Synthase (SS) in optimization-treated sample and all samples were confirmed through HPLC analysis as quantitative result and SDS-PAGE analysis as qualitative result. Subsequently the activities of SS were assayed through spectrophotometer. The results showed no significant different (p < 0.05) between SS activity with the trunk’s height. Developments of specific primers have been done by few researchers on sago palm. In this study, a pairs of PCR
primers were designed from cDNA library of sago palm and others starchy plants. The studies were initiated by total RNA isolation and RNA's conversion to cDNA. The cDNA integrity was confirmed using polymerase chain reaction technique using in house gene primer, called elf-F and elf-R. The cDNA was further amplified and sequenced. Primer with labeled ss1 was confirmed to be a specific primer for starch synthase as the BLAST resulted in percentage of similarities with Zea mays full length cDNA clone (79%), Zea mays starch synthase IIc precursor (68%), Triticum aestivum starch synthase IIc precursor (77%), and Oryza Sativa soluble starch synthase II-1 mRNA (77%). Characterization of SS in sago palm's trunk were further analyzed through Western blotting and the results has confirmed the presence of SS isoform at the size of 66.2 kDa, 45 kDa, 29 kDa, 26 kDa, and 17.7 kDa molecular mass. Although Northern blot analysis was failed, the specificity of the designed ssF1 and ssR1 primer was confirmed. Conclusively, this research has successfully identified the presence and size of SS isoform in sago palm's trunk and its activity was observed to be slightly gradually increased with the trunk's height.

Keywords: Starch synthase, Metroxylon sagu Rottb., Western blotting, Plawei, HPLC.
ABSTRAK
PEMPROFILAN DAN ANALISIS KANJI SINTASE PADA KETINGGIAN BERBEZA BATANG POKOK SAGU (METROXYLON SAGU ROOTTB.)

SDS-PAGE sebagai keputusan kualitatif. Aktiviti SS diekstrakan dengan menggunakan spektrophotometer. Keputusan menunjukkan tiada perbezaan (p<0.05) data diantara aktiviti SS dengan ketinggian batang pokok sagu. Penghasilan primer spesifik untuk pokok sagu telah dihasilkan oleh beberapa penyelidik sebelum ini. Dalam kajian ini, sepasang primer spesifik untuk PCR telah dihasilkan daripada perpusatakaan cDNA pokok sagu dan juga cDNA tumbuhan-tumbuhan berkanji lain. Kajian awal dimulakan dengan pengasingan semua RNA pokok sagu dan kemudian diterjemahkan kepada cDNA. Ketulinan cDNA tersebut dipastikan dengan menggunakan kaedah tindak balas polymerase berantai dan elf-F dan elf-R telah digunakan sebagai primer-primer “in-house”. Kemudian, cDNA diamplifikasi dan dijujukkan. Primer yang berlabel ss1 telah berjaya dipastikan sebagai primer spesifik untuk kanji sintase, dimana keputusan BLAST memberikan peratus kesamaan dengan rantaian penuh klon cDNA Zae mays (79%), prekursor kanji sintase IIc Zae mays (68%), prekursor kanji sintase IIc Triticum aestivum (77%), dan mRNA kanji sintase larut II-1 Oryza sativa (77%). Kajian mempro filkan SS diteruskan dengan membuat analisa Western blot. Keputusan daripada analisis Western blot mengesahkan kehadiran isofom SS pada berat molekul 66.2 kDa, 45 kDa, 29 kDa, 26 kDa, dan 17.7 kDa. Analisa Northern blotting tidak berjaya mencapai keputusan tetapi spesifikasi primer ssF1 dan ssR1 yang direka telah berjaya ditentukan. Keseluruhannya, kajian ini telah Berjaya mengesahkan kehadiran enzim SS didalam batang pokok sagu serta isofom-isofom enzim SS. Selain itu kajian juga telah berjaya menunjukkan aktiviti SS meningkat dengan perkadaran yang sedikit apabila kedudukan ketinggian pada batang pokok sagu meningkat.

Kata kunci: Kanji sintase, Metroxylon sagu Rottb., Western blotting, Plawei, HPLC
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iii</td>
</tr>
<tr>
<td>Abstrak</td>
<td>v</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xiv</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xvi</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xx</td>
</tr>
</tbody>
</table>

CHAPTER 1 - INTRODUCTION

1.1 Project Rationale                          1
1.2 Objectives                                  7

CHAPTER 2 - LITERATURE REVIEW

2.1 Sago Palm (*Metroxylon Sagu* Rottb.)       8
2.2 Starch Structure and Composition           11
2.3 Starch Biosynthesis                        13
2.4 Spectrophotometer Assay of SS              16
2.5 Western Blotting                           17
CHAPTER 3 - OPTIMIZATION OF PROTEIN EXTRACTION AND PURIFICATION METHOD IN STARCH SYNTHASE STUDY OF SAGO PALM (METROXYLON SAGU ROTTB.)

3.1 Introduction

3.2 Materials and Methods

3.2.1 Sampling

3.2.2 Enzyme Extraction Using Different Buffer

3.2.3 Determination of Total Protein Concentration

3.2.4 SDS-PAGE

3.2.5 Ammonium Sulphate Precipitation

3.2.6 Dialysis

3.2.7 Cold Acetone Precipitation

3.2.8 Enzyme Extraction Using Different pH

3.2.9 Spectrophotometer Assay of Starch Synthase

3.2.10 HPLC Analysis

3.3 Results

3.3.1 Enzyme Extraction from Three Different Buffers

3.3.2 Ammonium Sulphate Precipitation and Dialysis
3.3.3 Cold Acetone Precipitation 39
3.3.4 Detection of ADP 40
3.3.4 Enzymatic activity at different pH 43

3.4 Discussions 44
3.4.1 Extraction Buffers 44
3.4.2 Ammonium Sulphate Precipitation and Dialysis 47
3.4.3 Cold Acetone Precipitation 47
3.4.4 Detection of SS 48
3.4.5 Spectrophotometer Assay of SS 49

3.5 Conclusion and Future Direction 50

CHAPTER 4: PROFILING OF STARCH SYNTHASE ACTIVITY IN
PLAWEI GROWTH STAGES OF SAGO PALM
(METROXYLON SAGU ROTTB.) 51

4.1 Introduction 51

4.2 Materials and Methods 54

4.2.1 Sampling 54

4.2.2 Iodine-starch Complex Colorimetric Method and Moisture
Content Measurement 56

4.2.3 Enzyme Extraction 57

4.2.4 Determination of Protein Concentration 58
4.2.5 Ammonium Sulphate Precipitation and Desalting 58
4.2.6 SDS-PAGE 58
4.2.7 HPLC Analysis 57
4.2.8 Spectrophotometric Assay of Starch Synthase 57
4.2.9 Statistical Analysis 57

4.3 Results 60
4.3.1 Iodine-Starch Complex Colorimetric Method 60
4.3.2 Moisture Content Measurement 61
4.3.3 Enzyme Extraction and Protein Quantification 63
4.3.4 Detection of ADP 68
4.3.5 Spectrophotometric assay of SS 72

4.4 Discussion 75

4.5 Conclusion 80

CHAPTER 5: THE ESTABLISHMENT OF PCR-BASED SPECIFIC MARKER FOR STARCH SYNTHASE IN SAGO PALM (METROXYLON SAGU ROTTB.) 81

5.1 Introduction 81
5.2 Methodology 84
5.2.1 RNA Extraction 84
5.2.2 Spectrophotometric Measurement 85
5.2.3 Primer Design 85
5.2.4 Synthesis of First Strand cDNA

5.2.5 PCR Amplification

5.2.5.1 Polymerase Chain Reaction for cDNA Integrity

5.2.6 Native Agarose Gel Electrophoresis

5.2.7 cDNA Recovery From Agarose Gel

5.2.8 Sequencing of PCR product

5.3 Results

5.3.1 RNA Extraction

5.3.2 Synthesis of First Strand cDNA

5.3.3 PCR Amplification

5.3.4 Sequencing and analysis of PCR Product

5.4 Discussion

5.5 Conclusion and Future Direction

CHAPTER 6: WESTERN BLOT AND NORTHERN BLOT ANALYSIS OF STARCH SYNTHASE IN SAGO PALM (METROXYLON SAGU ROTTB.)

6.1 Introduction

6.2 Methodology

6.2.1 Samples
6.2.2 SDS-PAGE 106
6.2.3 Western Blotting 106
6.2.4 Color Development of Expressed Protein 107
6.2.5 Probe Design for Northern Blotting 108
6.2.6 Northern Blotting 108
6.2.7 Chemiluminescent Detection of Nucleic Acid for Northern Blotting 110

6.3 Results 111
6.3.1 SDS-PAGE 111
6.3.2 Spectrophotometer Quantification 112
6.3.3 Western Blotting 112
6.3.4 Northern Blotting 116

6.4 Discussions 117
6.4.1 Western Blotting 117
6.4.2 Northern Blotting 120

6.5 Conclusion 125

CHAPTER 7: GENERAL CONCLUSION AND RECOMMENDATIONS 127

8.0 REFERENCES

APPENDIX A: Standard Calibration Curve

APPENDIX B: Calculation for Enzymatic Activity of SS
APPENDIX C: Statistical Analysis For Spectrophotometer Assay

APPENDIX D: Statistical Analysis for Starch Content and Total Protein Measurement

APPENDIX E: Nucleotide Sequence for Primer Design
List of Tables

Table 2.1 The physiological growth stage of sago palm. 10
Table 3.1 The names of stages for each sample's label received from Craun Research Sdn Bhd. 27
Table 3.2 Protein concentration of crude enzyme of 5 g sago palm's trunk extracted using different buffers on a sample of sago palm’s trunk (AngauMuda stage). (n=3). 35
Table 3.3 Protein concentration of crude enzyme extracted from different growth stages of 5g samples of sago palm trunk using GBSS-buffer. (n=3). 36
Table 3.4 Concentrated protein recovered after ammonium sulphate precipitation and dialysis was performed on AngauMuda samples purified by GBSS-buffer. The 60% (w/v) of the ammonium sulphate precipitation gives the highest value of protein concentration. ((n = 3) 37
Table 4.1 Data of sago palm’s trunk at Plawei stage. 54
Table 4.2 The amount of starch in 1 g/mL of sago trunk. 60
Table 4.3 One-way ANOVA on starch content in 1 g/mL sample at different trunk’s part in Plawei stage of sago palms. 61
Table 4.4 Protein concentration of crude enzyme extracted from Plawei stage of sago palm trunk using GBSS buffer. 64
Table 4.5 Specific value of retention time, height, area, and molarities for each standard used in HPLC analysis. 68
Table 4.6 The amount of ADP produced during spectrophotometer assay of SS enzyme on three palms, specifically at Plawei stage. (n=3). 72
Table 4.7 Activity of SS observed at different heights of the sago palm at Plawei stage. (n=2). 73
Table 5.1 The sequence of primers that specifically designed for starch synthase. 86
Table 5.2 The reaction mixture for RNA cleanup prior to cDNA synthesis. 86
Table 5.3  The reaction parameters for the PCR analysis set up.  
Table 5.4  The optimized volume of PCR mixture for PCR reaction using ssF1 and ssR2 primers.  
Table 5.5  Molecular data of elf gene for PCR analysis  
Table 5.6  The optimized volume of PCR mixture for PCR reaction using elf-F and elf-R primers.  
Table 5.7  Purity and yield of the extracted RNA.  
Table 6.1  The optimal volume of sample’s mixture loaded into the formaldehyde gel’s well.  
Table 6.2  The volume of components in prehybridization solution.  
Table 6.3  The volume of components in Formamide hybridization solution.  
Table 6.4  The concentration of extracted RNA from each palm at different heights.  
Table 6.5  The occurrence of expressed SS within each sago palm. The square root symbol indicates the presence of respective sizes in the sample.  
Table 6.6  The percentage of different types of RNA in eukaryotic cells (Darling & Brickell, 1996).
List of Figures

Figure 1.1 The estimated area of sago in Sarawak in 2003 to 2007 (Courtesy of the Department of Statistics Sarawak (http://www.doa.sarawak.gov.my/statistik07.htm)).

Figure 1.2 The export of agricultural products by Malaysia in year 2007(http://www.doa.sarawak.gov.my).

Figure 2.1 Picture of Metroxylon sagu Rottb. at Plawei stage. Picture was captured at Bau (Singai area) district, Sarawak.

Figure 2.2 The molecular structure of amylose and amylopectin (Shaw, 1999).

Figure 2.3 Closed view of starch granules of sago palm. (A) The SEM micrograph of starch granules in native sago palm, 700x (Wong et al., 2005). (B) & (C) Optical microscope view of starch granule cultivated in mineral soil (Nozaki et al., 2004).

Figure 2.4 The three steps of starch biosynthesis in higher plants (Martin & Smith, 1995)

Figure 2.5 The classical enzymatic glucose reaction that has been applied in producing formula for enzymatic assay (Illanes, 2008).

Figure 2.6 The overall diagram of a HPLC system (Prichard et al., 2003).

Figure 3.1 Picture of a grater and grated sago pith samples.

Figure 3.2 The bands of crude protein extracted using three different buffers. (A) The samples extracted using the Wendel & Weeden buffer. (B) The samples extracted using GBSS buffer. (C) The sample extracted using SS buffer. Each lane contains 16 μL of the sample. Only one out of six samples is stained after SDS-PAGE process for SS buffer and GBSS buffer. Thus result shows only stained protein. The molecular standard of A is the Kaleidoscope prestained protein ladder (Bio-Rad).

Figure 3.3 The ammonium sulphate precipitation of samples extracted using Wendel & Weeden buffer. The yellow color above the tube is the precipitation of BSA in which might have affected the reading of the protein concentration measurement through BSA standard method.

xvi
Figure 3.4  Protein concentration of extracted sample from different growth stages after precipitated using cold acetone precipitation method.

Figure 3.5A  Graph shows the result of HPLC assay on precipitated enzyme extracted using GBSS-buffer at pH 4. There are six peaks was recorded in the graph above, indicated that the sample was not purified even after cold acetone precipitation. The highest peak at the retention time of 10.161 minutes indicated the level of ADPGlc in sample.

Figure 3.5B  Graph shows the result of HPLC assay on precipitated enzyme extracted using GBSS-buffer at pH 6.5. The number of peak that was recorded in graph was eight peaks, indicated that the sample was not purified even after cold acetone precipitation. The presence of ADPGlc was detected in peak number 4 (highest peak) with retention time of 10.116 minutes.

Figure 3.5C  Graph shows the result of HPLC assay on precipitated enzyme extracted using GBSS-buffer at pH 8. The number of peak that was recorded in graph was eight peaks, indicated that the sample was not purified even after cold acetone precipitation. The presence of ADPGlc was detected in peak number 4 (highest peak) with retention time of 10.105 minutes.

Figure 3.6  Figure above shows the progression activity of SS in spectrophotometric assay. The most favourable condition of buffer pH was detected at pH 8. The progression was measured in minutes of time against the enzyme activity. After 150 mins, starch synthase enzyme activity is dropped to negative value; therefore activity was only recorded within the 150 mins period of time. n=3.

Figure 3.7  Approaches in order to avoid browning effect. 3.7A; Sago trunk was chopped into large cube immediately at the sampling site. 3.7B&3.7C; Sample was stored in Falcon tube and wrapped in aluminum foil before stored in 4°C freezer.

Figure 4.1  A; The flat top shape of the chopped tree (Palm 1) indicated it was in Plawei stage. B; the worker is in the middle of slicing the trunk into disc form using a chain saw. C; fresh look of the inner trunk after it was sliced. D; the disc form of the trunk after slicing process using chain saw.

Figure 4.2  Fieldtrip on October 14, 2009 for the sampling of sago palm 2 and sago palm 3. A; complete image of the palms. B; an expert lab assistant of UNIMAS started to chop down the palm. C; measuring process on the diameter, circumference and length of the trunk.
Figure 4.3  The powder form of sample after grounded in mortar.

Figure 4.4  The percentage overview of total mass in 1 gram of the trunk of *Metroxylon sagu* Rottb. The number of 1 to 6 in the figure represents the sample from each high and part of sago trunk. Specifically, number 1 = base-centre, 1 = base-side, 3 = middle-centre, 4 = middle side, 5 = top-centre, and 6 = top-side.

Figure 4.5  Picture on part of sago trunk that was chose for enzyme extraction.

Figure 4.6  The bands of crude enzyme protein from six different part of palm 1. Lanes A to F in the Figure 4 represents the sample from each height and part of sago trunk. A = base-centre, B = base-side, C = middle-centre, D = middle side, E = top-centre, and F = top-side. Arrows indicates the protein bands.

Figure 4.7  The banding pattern of crude enzyme extracted from sago palm 2 (A) and sago palm 3 (B). Lane 7 and lane 8 contains the commercial protein, Bovine serum albumin, and α-amylase, respectively. Lanes 1 = base-centre, 2 = base-side, 3 = middle-centre, 4 = middle side, 5 = top-centre, and 6 = top-side. Arrows indicates the protein bands.

Figure 4.8  The faint bands detected on SDS-PAGE gel after undergo ammonium sulphate precipitation and desalting. The alphabet of A to F in the figure represents the sample from each high and part of sago trunk. Specifically, alphabet A = base-centre, B = base-side, C = middle-centre, D = middle side, E = top-centre, and F = top-side. Arrow indicates the protein bands at size of 66.2 kDa and 45 kDa.

Figure 4.9  Figure 4.9A- Figure 4.9F: Sample GBSS

Figure 4.10  The comparison of SS activity between three sago palms by estimated marginal means. Briefly the graph indicates that the difference in SS activity between palms was increased as the height of trunk moved up from base to the top, but insignificantly analyzed by ANOVA. (n=2).

Figure 5.1  Native gel electrophoresis of RNA extracted samples from sago palm. The volume of sample loaded into the well is 5 μL with 1% (w/v) agarose gel. All lane were loaded with three replications of sample; A = Base height, B = Middle height, C = Top height. Lane D is the 1 Kbp DNA ladder, indicating a positive control for this analysis.

xviii
Figure 5.2  Electrophoresis analysis shows that the single band in lane B signify construction of first strand cDNA was successful. The template RNA used to develop this cDNA was displayed on lane C. Lane A is the 100 bp DNA ladder.

Figure 5.3  Figure 5.3A shows the products of cDNA amplification before undergoes purification. Figure 5.3B is the purified PCR products obtained from sago palm cDNA after recovered from 1% (w/v) agarose gel electrophoresis analysis in Figure 5.3A. Lanes 1 and 3 indicates the purified PCR product at the size 376 bp molecular mass. Meanwhile 2 is the 1 Kbp DNA ladder.

Figure 5.4  Figure 5.4: Figure shows attempt for an optimization of ssF and ssR primers at specific temperature using gradient PCR. The template is cDNA developed from RNA’s sago palm. Smearing occurrence was observed within all samples, indicating the temperatures were not suitable for the primer. Specifically, number 1= 50°C, 2= 50.2°C, 3= 50.9°C, 4= 52°C , 5= 53.2°C , 6= 54.4°C , 7= 55.6°C, 8= 56.8°C , 9= 57.9°C , 10= 59°C , 11= 59.8°C, and 12= 60°C . Lane A is the 100 bp DNA ladder.

Figure 6.1  The diagram shows the overall process of Northern blotting. The pathway encompassed probe’s selection either from Oligonucleotides or cDNA and the labeling techniques are either using non-radioactive or radioactive (Trayhum, 1996).

Figure 6.2  Two types of protein transfer in Western blotting. Figure 6.2A: The outline of an electrophoresis transfer for a protein transfer system in wet transfer conditions (www.mitosciences.com). Figure 6.2B: The outline of an electrophoresis transfer for a protein transfer system in semi-dry transfer conditions.

Figure 6.3  The analysis of Western blot for palm 1. The number of 1 to 6 represents the sample from each height and part (center & side) of sago trunk. Specifically, number 1= base-centre, 2= base-side, 3= middle-centre, 4= middle side, 5= top-centre, and 6= top-side. Arrows indicates the location of the expressed SS along the lane.

Figure 6.4  The analysis of Western blot for palm 2. The number of 1 to 6 in the figure represents the sample from each height and part (center & side) of sago trunk. Specifically, number 1= base-centre, 2= base-side, 3= middle-centre, 4= middle side, 5= top-centre, and 6= top-side.

Figure 6.5  The analysis of Western blot for palm 3. The number of 1 to 6 in the figure represents the sample from each high and part of sago trunk. Specifically, number 1= base-centre, 2= base-side, 3=...
middle-centre, 4= middle side, 5= top-centre, and 6= top-side. Arrows indicates the location of the expressed SS along the lane.

Figure 6.6 Northern blotting analysis on sago palm 1 (A), sago palm 2 (B), sago palm 3 (C). No band was observed along all lanes, while marker is transferred completely.

Figure 6.7 The quality of the ss1 probe illustrated by the dot’s brightness. Numbers of 1 to 4 indicated the replicates of the prepared probe. Replicate for number 4 shows the probe’s concentration can be viewed at the lowest concentration of 30 pg.

Figure 6.8 The two phenomenon of RNAs base pairing. A: Intramolecular base pairing of short region. B: Intermolecular base pairing between different molecules of RNA (Darling and Brickell, 1994).
List of Abbreviations

ADP  Adenosine Diphosphate
AMP  Adenosine Monophosphate
ATP  Adenosine Triphosphate
G6PDH  Glucose-6-Phosphate Dehydrogenase
PEP  Phosphoenolpyruvate Kinase
NADP  Nicotinamide Adenine Dinucleotide Phosphate
PK  Pyruvate Kinase
HK  Hexokinase
HPLC  High Performance Liquid Chromatography
nm  Nanometer
SDS-PAGE  Sodium Dodecyl Polyacrylamide Gel
RNA  Ribonucleic Acid
DNA  Deoxyribonucleic Acid
cDNA  Complementary Deoxyribonucleic acid
mRNA  Messenger Ribonucleic Acid
miRNA  Micro Ribonucleic Acid
dNTP  Deoxyribonucleotides
PCR  Polymerase Chain Reaction
RT-PCR  Reverse transcriptase Polymerase Chain Reaction
%  Percentage
β  Beta

xx
\( \alpha \)  
\( ^\circ \text{C} \)  
mM  
m  
EDTA  
DTT  
mL  
HCL  
KOH  
CTAB  
PVP  
LiCl  
w/v  
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ml/min  
g/mL  
g/L  
Mg/mL  
mg/g  
nmol/mL\(^{-1} \)  
g  
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Alfa  
Degree Celsius  
MilliMolar  
Meters  
Ethylenediaminetetraacetic Acid  
Dithiothreitol  
Millimeters  
Hydrochloric Acid  
Potassium Hydroxide  
CetyltrimethylammoniumBromide  
Polyvinylpyrrolidone  
Lithium Chloride  
Weight per Gram  
Volume per Volume  
Millimeters per Minutes  
Gram per Millimeter  
Gram per Liter  
Milligram per Millimeter  
Milligram per Gram  
Nanomole per Milliliter  
Grams  
Milligrams  

xxi