GWENT GROUP
ADVANCED MATERIAL SYSTEMS

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With compliments

Dr Guido Drago
Managing Director

Guido Drago

Mission Statement

“ To become the World Leader in the Stabilisation of Proteins and Enzymes for Industrial Use”

Company Objectives

The mission of AET is to build a successful, growing, profitable business, based upon its expertise in the field of protein biotechnology. The aims of the company are two fold:

- To be the leading company world-wide in the field of protein stabilisation technology
- To create a “1 Stop Shop” for the development and production of stabilised biosensors

To achieve these aims AET has ambitious plans for the development of its technology and for the growth of its business. This is further enhanced by the obvious synergy with its sister companies **Gwent Electronic Materials Ltd.** and **Gwent Sensors Ltd.**

The Company

Applied Enzyme Technology Ltd (AET) is a company with core expertise in protein stabilisation. Application of our expertise and patented technology has resulted in the successful stabilisation of many enzymes spanning a wide variety of applications. Our research and development programme continues to generate and characterise novel tools for the stabilisation of labile enzymes of interest in many different industries including, the pharmaceutical and environmental industries.

AET has licensed its stabilisation technology into companies world-wide including, Amersham Pharmacia Biotech AB, Yellow Springs Instruments Inc, Biocatalysts Ltd., Symbollon Inc. and Capital Controls Ltd.

Applied Enzyme Technology Ltd. was established in 1994 to exploit a portfolio of intellectual property generated from research programmes initiated in the School of Biochemistry and Molecular Biology at the University of Leeds.

AET was granted a SMART 1 Award from the Department of Trade and Industry in 1995 to develop enzyme based kits with novel dye detection systems. As a straight follow on from the 1st SMART award, a second stage SMART 2 grant was won in 1996 to extend the range of enzyme stabilisation systems.
In 1996 AET was invited to participate as a full partner in an EU funded project, and since then has been involved in 4 subsequent EU funded projects and one as project Co-ordinator. The latest involvement being as a subcontractor on a project that started at the beginning of 2001.

In June 2001 a leading electronic sensor materials company, Gwent Electronic Materials Ltd. part of the Gwent Group, invested heavily in AET and as a consequence AET relocated near Cardiff in South Wales. AET now runs as an independent subsidiary of Gwent Electronic Materials Ltd. where our core business remains the same.

The synergy between the two companies brings together the expertise in enzyme stabilisation of AET and the expertise in the design and production of specialist electronic sensor materials attached to **Gwent Electronic Materials Ltd. Gwent Sensors Ltd.**, another company within the Gwent Group, specialises in development of disposable sensor technology. With access to all the above technology, all 3 companies are in the in the unique position to be able to offer a "**1 Stop Shop**" for the development and

production of biosensors. This has been taken one step further, as the Gwent Group can now provide the complete biosensor package including specialised instrumentation due to close collaboration with specialist instrumentation development companies

Most recently AET has been awarded A BIOWISE Demonstrator Project from the DTI.. This started In January 2003 with the remit of manufacturing disposable ammonia sensors for water pollutant monitoring, (See biosensors section for more detail).

Platform Technologies

Protein Stabilisation Technology

This is the core technology of AET Ltd. Enzymes, antibodies and pharmaceutical proteins have been stabilised to thermal, chemical and proteolytic degradation after processing. This methodology involves the modification of the protein microenvironment, which essentially leaves protein structure unaltered while preserving high biological activity. Enzyme preparations have been successfully dehydrated by lyophilisation or air drying routes while retaining high activity. Solution stabilisation of enzymes and antibody conjugates has also been successfully demonstrated. AET provides these services for a number of different enzyme systems, with the capacity to apply this technology to a multitude of putatively useful enzyme targets.

Novel Biosensors

Stabilisation of biomolecules used to produce specific responses in biosensors is a highly specialised area of protein stabilisation. AET has been particularly successful in technology transfer from experimental results to commercially available devices, by improving the stability of an enzyme system.

Biocatalysis and the PolyEnz™ Process

The basis of the PolyEnz process, involves the immobilisation of pre-stabilised enzymes. This process has been developed to produce stabilised biocatalysts for biosynthetic processes, biodegradation and waste treatment systems, as well as the biotransformation and production of drugs and their precursors.

Formulation Troubleshooting

The area of formulation, whether applied specifically to a protein drug, an assay, a diagnostic reagent or a traditional pharmaceutical, is an area involving specialised knowledge of interactions occurring in solution or dehydrated preparations. AET possesses a wealth of experience in this field. As well as providing a world-wide service to assist in formulations, we test the stability of the interim and final products produced.

Areas for Potential Collaboration

Protein and Enzyme Stabilisation

Co-development of stabilised protein and enzyme systems for product enablement
Diagnostic development and stabilisation of protein /enzyme reagents, including antibodies and conjugates
Feasibility studies for stabilisation of new proteins
High activity dehydrated proteins and enzymes

Protein and Peptide Therapeutics

Formulation troubleshooting to identify the optimal formulation for specific therapeutics
Stabilisation of proteins and peptides
Development of delivery systems for stabilised drugs
Carrier proteins or adjuvants for peptide vaccines

Biocatalysts and Immobilised Systems

Development of stabilised biocatalysts
PolyEnz Process
Immobilisation systems

Biosensors

Development of stabilised biosensors
Design of stabilisation systems for biomolecules used in biosensors
Biomimetic sensors
Immobilisation procedures for biosensors

Other Applications

Stabilisation of cells
Stabilisation of enzymes in foodstuffs and beverages
Stabilisation of enzymes in waste treatment and bioremediation
Stabilisation of enzymes in animal feeds.

Products

Protein Stabilisation Kits

The enzyme/protein stabilisation kit manufactured and marketed by Applied Enzyme Technology Ltd. is the result of over 9 years of expertise in the field of protein stabilisation. The data supporting the composition of these kits has been accumulated from the combination of 2 Smart awards, 4 European Commission funded projects and a multitude of client contract research projects.

4 kits are currently manufactured by AET. These contain 9 stabiliser formulations each, and have been designed to stabilise enzymes in the dry state (STKED), enzymes in solution (STKES), antibodies (STKAB) and pharma approved formulations (STKPH).

Applied Enzyme Technologies Protein Stabilisation Kit (product numbers STKED, STKES, STKAB, STKPH)

These formulations have been designed to meet the majority of the stability issues encountered by clients working both in industry and academia. The kit stabilises the majority of enzymes and proteins including both conjugated and unconjugated antibodies both in liquid formulations and dry formulations. The flexibility of the kit allows the investigator to use their own buffer systems, pH ionic strength etc. The formulations are made up in a concentrated form thus allowing for different buffer compositions and pH criteria. To date AET has stabilised over 50 enzymes/proteins in both liquid and dry forms.

Stabilised Proteins

The enzyme/protein groups studied and successfully stabilised are listed below:

- Horseradish Peroxidase-Conjugated Antibodies
- Alkaline Phosphatase conjugated antibodies

- Esterases
- Hydrolases
- Kinases
- Lipases
- Luciferase
- Oxidases
- Oxidoreductases
- Peroxidases
- Phosphatases
- Proteases

Most of the enzymes listed above have been stabilised individually. However, certain enzymes have been successfully combined to made stable working enzyme cocktails.

Most contract research has led to the generation of stable enzyme formulations from between 50 days to over 18 months at temperatures up to 50°C. The data generated by AET addresses both the issues of increased shelf life and the operational stability of the enzyme system in question.

Industrial Applications

Listed below are some of the examples in which our stabilisation technology is currently being used and can be used in the future.

- Biocatalysis Industry
- Bioremediation Industry
- Biosensor Industry
- Cosmetics Industry
- Diagnostics
- Human Healthcare
- Hygiene Industry
- Pharmaceutical Industry

For further information on pricing. **Contact AET.**

Bulk Stabilisers

AET produces and supplies clients with bulk quantities of final stabiliser formulations. We have the production facilities to handle up to hundreds of litres or hundreds of kilos of stabiliser formulations.

For further information on pricing. **Contact AET.**

Alcohol Oxidase (Hansenula polymorpha)

Unstabilised Form

State: Liquid
Specific activity: 450units/ml
Amount available in stock 100,000 units
Stored at -70°C
Stable for years

Stabilised Form

State: Dry stabilised powder
Available on request
Stability data: Stable for at least 162 days at 37°C in dry state

For further information on pricing. **Contact AET.**

Contract Research

Does the activity or shelf life of your protein cause problems with your final product formulation?
If so, AET can provide the answer.

Brief company Outline

AET is fast becoming the leading company world-wide in the field of protein stabilisation. Our research is currently assisting:

- pharmaceutical companies
- diagnostic companies
- biosensor manufacturers
- enzyme producers
- agricultural / food companies
- biotechnology companies
- instrument manufacturers

Solutions

AET are providing solutions to:

- improve protein stability
- increase product shelf life
- remove the need for refrigeration
- improved operational stability
- increase activity of your preparation
- reduce manufacturing costs

Costs

Each feasibility study will be a minimum of 10 days at a fixed daily rate.

Other Services

Rapid Stabilisation Screening Service

Applied Enzyme Technology Ltd. possesses the expertise to stabilise any protein or enzyme, in either the wet, dry or immobilised states. Our screening service combines our wealth of scientific expertise with the latest technology to enable rapid and detailed screening of the vast polymer/polyalcohol library we have at our disposal.
The following steps will be followed to ensure the successful screening of your protein:

1. An initial enzyme assay troubleshooting service will be used to design the optimum assay conditions for your enzyme, including the design of novel enzyme assays if required.
2. A preliminary stabiliser screen will determine the optimal stabiliser combination required to increase the stability of your product.
3. A detailed optimisation of the stabilisation formulation which best suits your product.

   The parameters investigated will include:

   - Choice of optimal stabiliser concentration.
   - Optimal buffer.
   - Optimal ionic strength of chosen buffer
   - Optimal pH.
   - Any unique additives required for improved stabilisation of the protein under investigation.

   The more detailed determination of optimal stabiliser concentration is achieved using a combination of analytical techniques. These include standard and novel enzyme activity assays using factorial experimental protocols, specially designed for rapid throughput with the latest plate reading technology. A combination of agarose gel electrophoresis and high resolution isoelectric focusing are also employed to determine the exact point at which protein/stabiliser interactions no longer convey any improved enzyme activity.

4. Finally, we will provide you, the client, a complete and detailed breakdown of our findings in an easily interpretable package. The screening protocol is highly standardised and contains built in quality control checks at all stages in the process, from experimental design to data analysis.

To get the most benefit from our stabilisation screening service, we recommend that an initial screen, duration 10 days. The requirement for more detailed screening can be discussed at this point.

Formulation Troubleshooting

With 2 granted patents on stabilisation of dry formulations and two patents on stabilisation in solution and sugar sensor devices, respectively, Applied Enzyme Technology Ltd (AET) is at the cutting edge of the field in protein stabilisation.
AET Ltd has been working in the area of protein stabilisation particularly enzymes for several years and has a proven record of success.

- Several licences to the patented technology, with marketed products from third parties and products under development
- A stabilised acetylcholinesterase product being developed towards commercial release
- Several successful feasibility studies carried out on a variety of proteins
- Proven stabilisation of over 50 different enzymes
- Development of the PolyEnz™ Process for stabilised biocatalysts

AET Ltd has commercial know-how developed over the last 10 years that has been found to be useful in the area of pharmaceutical formulation. The experience of the company has enabled several problems associated with formulation to be identified and successfully overcome for third party contractors.
Formulation troubleshooting includes:

- Development of rapid, simple assay protocols where possible (standard assay conditions for recombinant enzyme based pharmaceuticals have already been completed)
- Development of formulations to solve problems encountered during development (e.g. solubility problems, stability changes due to scale up to commercial levels, inactivation upon dilution)

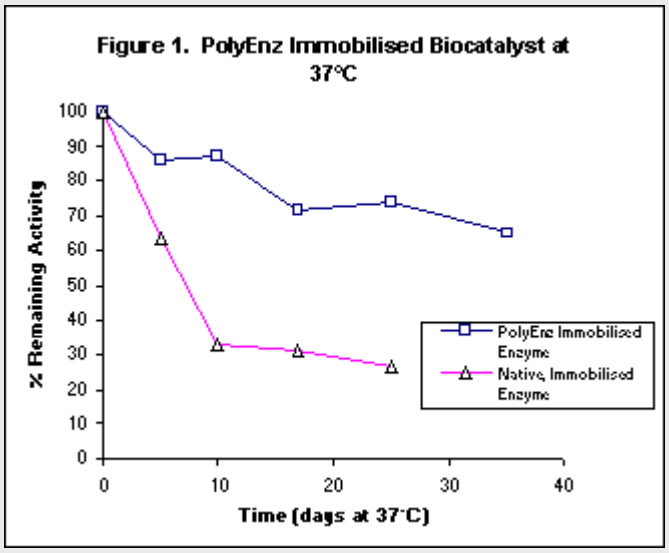
- Formulations for storage or shelf-stability increases
- Formulations for lyophilisation
- Identification and removal of incompatibilities in formulations (e.g. inactivation of proteins by the buffer system used, incompatibility of excipient with the active involved)

The PolyEnz Process

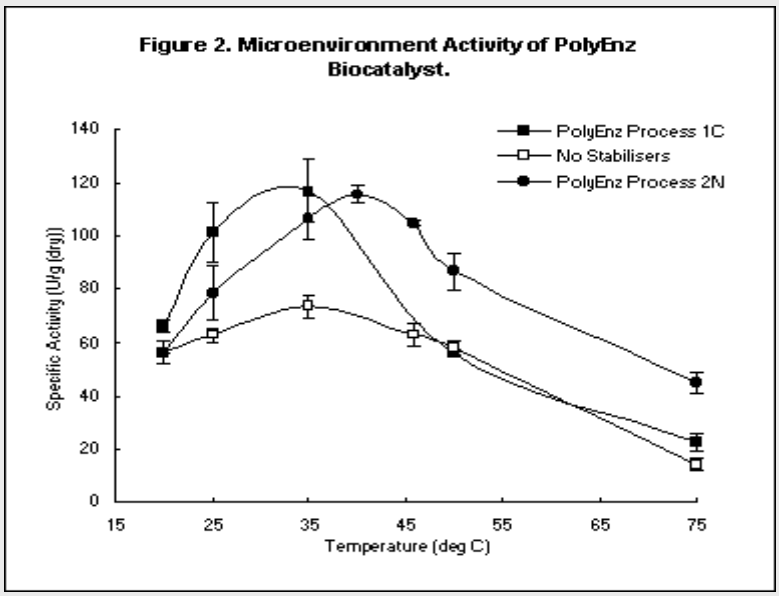
This process produces pre-stabilised enzymes which can then be immobilised onto or into a variety of matrices for the production of stabilised biocatalysts for production of pharmaceuticals, chemical synthesis, biotransformations and bioremediation.

The process has demonstrated on a solid activated particulate support, using an oxidoreductase enzyme as the immobilised biocatalyst.

Significant improvements of the shelf-stability of the immobilised biocatalysthave been recorded.

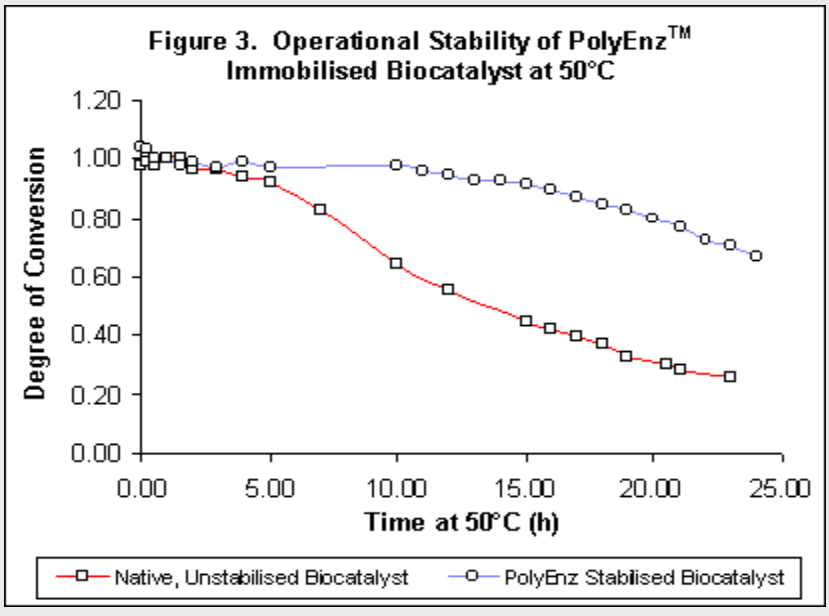


The PolyEnz Process also changes the microenvironment of the immobilised biocatalyst, which can lead to higher enzyme activity levels, figure2. The effect of this would be to improve production and potentially to improve efficiency of catalysis.



It is well known the microenvironment of enzymes often has a significant part to play in activity, stability and catalytic properties. Such microenvironment manipulations are at the heart of AET expertiseand are likely to prove crucial to the performance and stability of PolyEnz immobilised enzymes.

Figure3 shows the effect of the PolyEnz Process on the operationalstability of an oxidoreductase biocatalyst at an elevated temperature of 50°C, compared to the same biocatalyst immobilised in its native form. The improvement of activity retention is clearly apparent,leading to improved productivity and a reduction in costs overall.



Applications of Stabiliser Technology

Protein Stabilisation Technology

Enzymes are used in an increasing number of application areas including: detergents, food processing, household products, textile processing, manufacture of pharmaceuticals and chiral molecules, brewing, environmental and clinical assay kits, particularly as labels in immunological ELISA tests and biosensors. Many of the enzymes used in the described areas are unstable and often require refrigeration to maintain activity or some sort of stabilisation matrix is used.

Protein stabilisation technology has been patented and is being commercialised through AET. The technology has been developed to stabilise proteins, particularly enzymes in both the dry state and in solution for long term shelf-storage. The system is currently employed in a number of commercially available products and is under evaluation at a number of industrial and academic laboratories throughout the world for the production of other viable biosensors and diagnostic kits and reagents. We have produced methods for enzyme stabilisation which are currently in the formulation stage and in use in a number of applications. AET has also demonstrated very high operational thermal stability for immobilised biocatalysts, which is applicable to a multitude of industrial areas including the biocatalytic and biosensor industries. A significant amount of the business generated by AET is also based upon our extensive and highly valuable know-how that has been accumulated by AET in the areas of system optimisation and protein stabilisation. As a direct result of this generated knowledge, AET has recently launched a protein stabilisation kit, which enables clients to troubleshoot their own stability problems.
See Case Studies on:

- See website **Biosteriliser** case study

Speciality Enzyme Production.

AET has the experience and know how, coupled with excellent academic and industrial contacts to produce a number of speciality enzymes for use in specific biotechnological applications.
In addition, the company has the potential to interface between industrial and academic outlets in order to facilitate the production of certain specialised enzymes in a customised manner for a diverse number of application areas.
See Case Studies on:

- See **Alcohol Oxidase** (products)

Stabilisation of Biosensors

The Biosensor industry is considered a multi-billion dollar business and as enzymes are at the core of a working biosensor, enzyme stability issues are always at the forefront of producing a stable and reproducible biosensor.

AET has demonstrated the stability of a multitude of enzymes in working biosensor systems including, lactate oxidase, malate dehydrogenase, alcohol oxidase and glutamate oxidase All show increased stability characteristics in biosensors. An alcohol biosensor, stabilised using our patented technology, is currently produced under licence by Yellow Springs Instruments Inc. of Yellow Springs, Ohio.

A leading water treatment company has also recently licensed AET's technology to enable rapid measurement of water quality in the field.

Sensor Technology for Rapid Environmental Ammonia Monitoring (STREAM)

Lead partner: Applied Enzyme Technology Ltd
Partners: Gwent Electronic Materials Ltd, Environment Agency
Biotechnology application: Monitoring

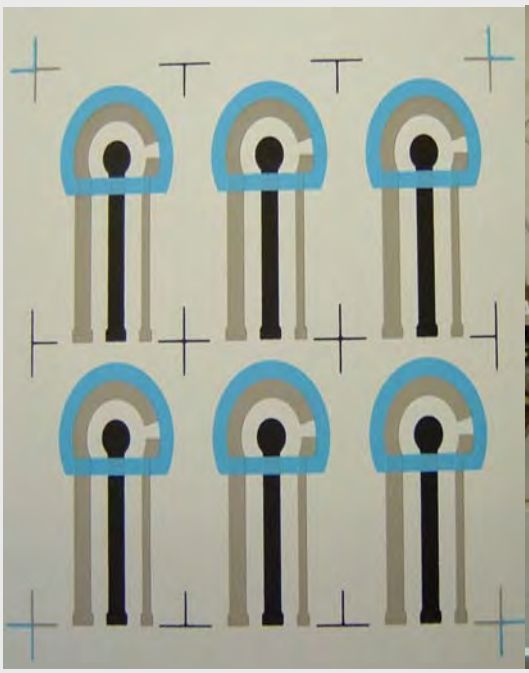
A BIO-WISE Demonstrator Project grant of £188,093 has been offered to a two-year project to demonstrate the use of a novel, enzyme-based biosensor to monitor ammonia levels in effluent and sewage.

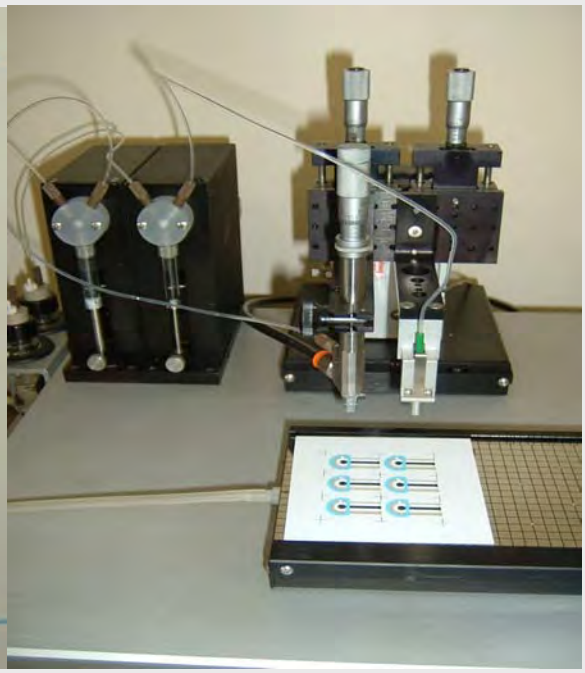
Ammonia is a significant pollutant of water and is monitored in rivers in England and Wales by the Environment Agency (EA). There are significant potential benefits to be gained by industry from a quick, low-cost, simple and sensitive test that can be carried out at the site of sampling. This project aims to demonstrate such a test, with the ultimate goal of developing a handheld instrument.

The biosensor will consist of modified screen-printed carbon electrodes (SPCE) that are coated with the enzyme, glutamate dehydrogenase. This enzyme catalyses ammonium ions and brings about an electrochemical change. This is detected by the SPCE to produce an electronic signal relating to ammonium ion concentration.

This project is led by Applied Enzyme Technology Ltd, an SME with experience in enzyme stabilisation and protein immobilisation. It is collaborating with Gwent Electronic Materials, also an SME with a background in sensor technology, to produce a stable biosensor. The role of the EA in the project will be to provide guidance on measurement specifications and field trials, and ultimately to be an end user of the technology.

The potential uses of the biosensor for self monitoring of ammonia levels are significant. Independent testing is required for the paper, textile and leather industries to ensure that only acceptable levels of bleaching agents enter local watercourses. Self-monitoring may also be required in the agricultural industries, as a result of nitrate run-off, and in the fish-farming industry where effluent from these farms directly affects river ecosystems. Other industries (eg chemical, petrochemical, pharmaceutical and metallurgic) also have high levels of ammonium ion production during industrial processes. The effluent from these industries must be monitored and treated prior to discharge into local river networks. Therefore, with a reliable test method, there will be a worldwide market for an ammonia biosensor.





Case Studies of Dry Stabilised proteins see website:

- **Alcohol Oxidase**
- **Lactate Biosensors**
- **Glutamate Biosensors**

Diagnostic Test Kits

AET's stabilisation technology was initially generated as a result of our first SMART award. There was so much interest in the stabilisation technology that a 2nd SMART award based on stabilisation technology was awarded. Thus much of AET's expertise and stabilisation technology has been applied to the use of antibodies and enzymes into diagnostic kits. AET has carried out much work with clients on optimising the operational and shelf life stability of diagnostic kits in the food and hygiene industries, as well as stabilising a multitude of conjugated antibodies as labels for diagnostic kits.

Case Studies see website:

- **IgG-HRP**
- **Alkaline Phosphatase**
- **Horseradish Peroxide in solution**
- **Horseradish Peroxide Dry Stability**

Stabilisation of Proteins in Solution

The solution stabilisation protocols developed at Leeds appear to be generic in conferring thermal, chemical and proteolytic stability to a wide range of different proteins.

The protocols developed use combinations of soluble polymers and additives incorporated into the buffers used for protein stabilisation. Particular Features Include:

- Extended Shelf Life of Proteins in Solution
- Promotion of Operational Stability
- Protection Against Dilution Inactivation
- Protection Against Proteolytic Degradation

Proteins Stabilised in Solution

Protein	Length of Stability	Storage Temperature (°C)
Acetylcholine Esterase	180 mins	55
Alcohol Oxidase	30 mins	56
Alkaline Phosphatase (Bacterial)	476 days	4
Anti-TSH-HRP Conjugate	240 mins	58
Bovine Alkaline Phosphatase	60 mins	62
Catalase	160	60
Creatinase	5 days	37
Creatininase	5 days	37
Cutinase	180 mins	88
Glucose dehydrogenase	21 days	37
Glucose Oxidase	246 days	22
Glycerol kinase	120 mins	52
Glycerol phosphate oxidase	330 mins	40
Goat anti-human IgA Alkaline Phosphatase	180 mins	55
Horseradish Peroxidase-4	180 days	40
Horseradish Peroxidase-4	24 hours	69
Lactate Dehydrogenase	5 days	37
L-Glutamate Dehydrogenase	100 days	45
Lipase	14 days	25
Luciferase	50 mins	45
Penicillin G Acylase	20 mins	60
Rabbit anti-DNP HRP Conjugate	24 hours	58
Rabbit anti-Human IgG-HRP Conjugate	221 days	25
Recombinant Horseradish Peroxidase	80 days	50
Sarcosine Oxidase	5 days	37
Savinase	28 days	40
Savinase	21 days	50
T4-HRP Conjugate	230 mins	67
Thermophilic Glutamate Dehydrogenase	30 days	40
Trypsin	21 days	50

Stabilisation of Dehydrated Proteins

The stabilisation technology discovered and developed at Leeds appears to be generic in its ability to confer stability to proteins in the dehydrated state.
The technology uses combinations of soluble additives and polymers premixed with the protein prior to the drying step.
Particular Features Include:

- Protection Against Activity Loss During the Drying Step
- Extended Shelf Life of the Dry Product
- Stabilisation of Immobilised Enzymes
- Stabilisation of Biosensors
- Effective in Dry Chemistry Formats

Proteins Stabilised in the Dry State

Protein	Length of Stability (Days)	Storage Temperature (°C)
Acetylcholinesterase	76	37
Alcohol Oxidase	162	37
Catalase	22	37
Cholesterol Oxidase	16	37
Choline Oxidase	15	37
Diaphorase	150	37
Fructose Dehydrogenase	155	4
Fructose Dehydrogenase	155	25
Galactose Oxidase	17	37
β-Galactosidase	65	25
Glucose Dehydrogenase	13	37
Glucose-6-Phosphate Dehydrogenase	150	37
Glutamate Dehydrogenase	183	22
Glycerol-3-Phosphare Oxidase	37	15
Hexokinase	150	37
Horseradish Peroxidase	50	37
β-Hydroxybutyrate Dehydrogenase	36	37
Lactate Dehydrogenase	190	25
Lactate Dehydrogenase	98	37
Lactate Oxidase	300	37
Lactate Oxidase	300	50
Malate Dehydrogenase	20	37
NADH	182	22
Pyruvate Kinase	150	37
Serine Protease	56	66
Uricase	10	37

Existing Intellectual Property Rights Granted Patents and Patent Applications owned by AET

Dry Stabilisation Cationic Enzyme Stabilisation 1:

Patent application WO/090/05182; Issued in the US in August 1993 under no. 5240843; Granted in Australia October 1993 under no. 637776; Granted in Canada in January 1996 under no. 2040815; 'Patent application PCT/GB89/01346 granted July 1993.
The Territory means the countries listed below:
WO/090/05182

1. United States of America
2. Canada
3. Australia

PCT/ GB89/01346

1. United Kingdom
2. Austria
3. Belgium
4. Germany
5. France
6. Italy
7. Netherlands
8. Sweden

Dry Stabilisation Anionic Enzyme Stabilisation 2:

Patent application WO/091/14773; 'Patent application PCT/GB91/00443 granted November 1997.
The Territory means the countries listed below:
PCT/ GB91/00443

1. United Kingdom
2. Germany
3. France
4. Sweden.

Solution Stabilisation:
Enzyme Stabilisation 3

Patent application WO/095/10605; issued in the US in October 2000 under no. 08/624585 ‘Patent application PCT/GB94/02180; awaiting EPO examiners report
The Territory means the countries listed below:
WO/095/10605

1. United States of America

PCT/GB94/02180

1. Austria
2. Belgium
3. Switzerland
4. Germany
5. Denmark
6. Spain
7. France
8. United Kingdom
9. Greece
10. Ireland
11. Ireland
12. Italy
13. Luxembourg
14. Monaco
15. Netherlands
16. Portugal
17. Sweden

Contact Details

Should you require any further assistance on the information contained in this website, then please do not hesitate to contact us at any time.

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