



Bioclone Australia Pty Limited

CONTRACT SERVICES

RESEARCH & DEVELOPMENT

&

MANUFACTURING

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Bioclone's Quality Management System certified to ISO 9001, ISO 13485, GMP and CE Mark

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Contract Services Research & Development & Manufacturing

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Bioclone also markets a large range of immunoassay kits - IRMAs, RIAs, ELISAs and allergen products.

For further information, please contact Bioclone or visit Bioclone's website.

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BIOCLONE

BIOCLONE AUSTRALIA PTY LIMITED

Background:

Bioclone is an immunodiagnostic company, established in Sydney, Australia in 1981. Since 1996, Bioclone has been a wholly-owned subsidiary of Hitachi Chemical Co., Ltd., moving to a purpose-built facility in 1997.

Bioclone is a quality-driven company with quality assurance certifications (current editions) including those for:

- ISO 9001
- ISO 13485
- GMP
- CE Mark

Furthermore 10 of the ELISA range products have US FDA clearance with 510 (k) approval to market. A number of products have also been registered through the Japanese Ministry of Health.

Bioclone manufactures immunodiagnostic kits and reagents for the medical and research laboratory markets, mainly covering testing for pregnancy, fertility, growth markers, thyroid functions, anaemia, allergy, neonatal screening, urinary iodine measurement and *Chlamydia* diagnosis.

The core product range is directly distributed domestically and through a network of pro-active distributors globally – either under the Bioclone label or by OEM arrangement. There is also scope for biological raw material supply.

Bioclone's contract services focus on Research & Development and Manufacturing and accomplishes this by utilising its strategic alliances and research and commercial contact base.

Bioclone has the capacity for small to large biotech activities, including platform ELISA technology, which is underscored by cost-effective development and production. Bioclone's manufacturing capabilities include freeze drying, microtitre plate coating, monoclonal and polyclonal antibody production and purification, iodination, biotinylation, other conjugations and antibody manipulation. Additionally it includes immunodiagnostic production from components to full kit manufacturing.

Bioclone encourages co-operative joint partnering for new projects with part of the current expansion strategy being focussed on strategic alliances as mentioned above, particularly in relation to Contract work (R & D or manufacturing) and product range extension, including unique / niche assay development.

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Immunoassay Development Made Easy

Introduction:

To shorten the time to market, Bioclone has available a large range of monoclonal antibodies presented as either unpurified or purified antibodies.

This range is augmented with commercially proven antibody pairs, validated in either IRMA or ELISA formats.

Streptavidin-Peroxidase Development kits containing tried and tested ready-to-use reagents can also be sourced from Bioclone to aid development plans.

As a further illustration of Bioclone's commitment to simplifying immunoassay development, other diagnostic-related products including polyclonal antisera from various hosts are also included as inventory items or can be produced and purified to specifications.

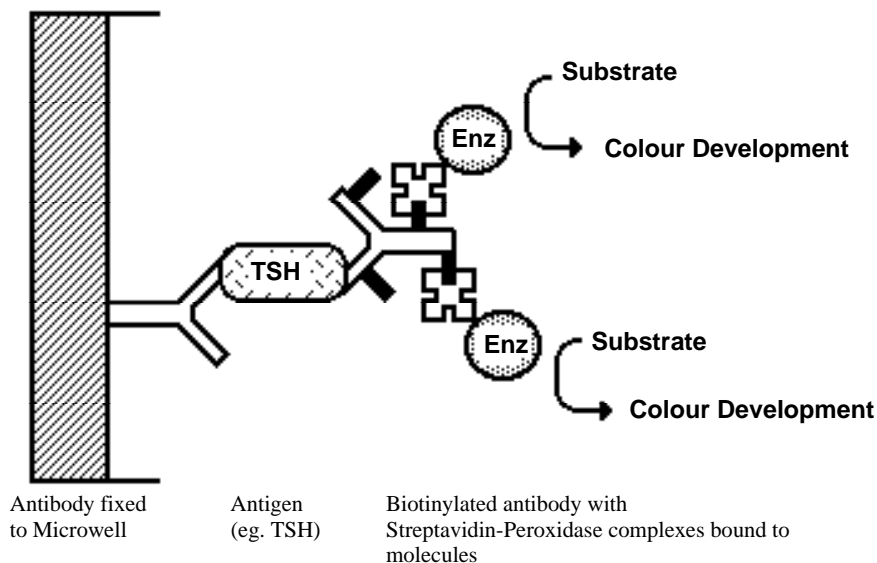
Whether Bioclone supplies customer needs directly or collaborates by providing contract services over the spectrum of immunodiagnostic activities, including for example immunoassay kit development and manufacture, large-scale microtitre plate coating or antibody production including bulk production of monoclonals, the company has a flexible outlook geared to a commitment to any client's needs.

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ELEGANCE ELISA SYSTEM

AMPLIFIED ELISA SYSTEM



- * **Amplification:** Biotin-Streptavidin linkage for increased dynamic range and sensitivity.
- * **Monoclonal/Polyclonal Pair:** for high specificity and assay speed.
- * **Precision:** offered by sandwich assays.
- * **96-Well Plate:** comes in strip format to cater for large or small runs.
- * **Colour-coded Reagents:** for easy identification and improved QC.
- * **Ready-to-use Components:** with extended shelf-life.
- * **Non-Isotopic:** utilizing enzyme technology with long shelf-life.
- * **One Component Substrate:** 3,3',5,5'-tetramethylbenzidine (TMB).
- * **Open System:** for use with all non-dedicated instrumentation.



Assay Development Guide:

Bioclone reagents and protocols that can prove helpful in assay development and optimization are noted below:

1. Bioclone has two, stable, one-component, liquid TMB chromogenic substrate formulations available (depending on sensitivity requirements) for peroxidase conjugates: a high activity formulation (Ref# ETMB5 - 50 ml; ETMB25 - 250 ml) and a normal activity formulation (Ref# TMBB5 - 50 ml; TMBB25 - 250 ml). Both products produce low NSB and have a long shelf-life. As one-component liquids, each formulation is easy to handle. Note that Bioclone also has an OPD tablet / Substrate Buffer combination available (Ref# EST4 - 4 x tablets / Ref# ESB20 - 20 ml). For alkaline phosphatase conjugates, a stable, one-component, liquid p-NPP reagent is available (Ref# CSL96 - 10 ml). Bulk supplies of each of the above are available on request.
2. Bioclone has a stabilized streptavidin-peroxidase formulation. The reagent (Ref# EAMP10.480 - 50 ml) provides a high concentration of streptavidin-peroxidase with low NSB and long shelf-life. It is ready-to-use against biotinylated proteins and coloured (violet) for ease of identification and use. Dilutions of the reagent can be made, if required, but stability is suitably maintained if dilutions are in the requisite stabilizing buffer. (The buffer is available only on request).
3. Bioclone has a stabilizing ELISA Plate Blocking Buffer (Ref# EBLOCK - 1000 ml) which not only provides low NSB but also has the ability to produce dry coated plates with long stability (usually a shelf-life of greater than 18 months). This means larger batch runs can be produced for better assay consistency. It is ready-to-use.
4. Bioclone produces anti-mouse IgG coated microwells (Ref# T4A96) and anti-rabbit coated magnetizable particles (Ref# SEP1 - 13 ml; SEP2 - 26 ml), each useful in capturing their target antibodies (mouse or rabbit respectively) as part of a (competitive) assay or screening protocol.
5. *Adjunct assay reagents include:*
 - ELISA Wash Concentrate (Ref# EWC5 - 250 ml; EWC800 - 450 ml) - a 15-times wash concentrate which is very stable and efficiently removes NSB. Dilute before use.
 - ELISA Stopping Solution (Ref# ESS25 - 130 ml) - used for stopping either OPD or TMB reactions. It is ready-to-use.
 - ELISA Stabilizing Stop Solution (Ref# ESCL25 - 130 ml) - particularly useful if there are delays before reading a TMB-filled plate after stopping. It is ready-to-use.

As noted elsewhere, Bioclone, under contract manufacture, can carry out antibody purifications, biotinylations, iodinations, conjugations, couplings to magnetizable particles and microwell coating. Contract R&D is also possible for larger projects (including monoclonal antibody development and assay development).

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ELISA Accessory Reagents

The following pages contain Technical Data Sheets on core componentary and other reagents that have been discussed elsewhere within this document.

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Technical Data Sheet:

ELEGANCE Streptavidin-Peroxidase Development Kit

Reference#:	ESDK480	Component Ref.#	Amount / bottle	No. of bottles
	Amplification Reagent	EAMP10.480	50mL	1
	Substrate Buffer (H ₂ O ₂)	ESB20	20mL	2
	Substrate Tablets (OPD)	EST4	4x4mg	2

Description: Streptavidin-peroxidase amplification substrate system designed to detect biotinylated reagents in immunological detection systems.

Contents: *Amplification Reagent:*
Streptavidin-peroxidase in a buffered solution containing bovine serum albumin and a violet dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready-to-use.
Substrate Buffer:
Urea Peroxide (H₂O₂) in a citrate-phosphate buffer. Contains thiomersal, 0.01% w/v.
Substrate Tablets:
Ortho-phenylenediamine (4mg each) with inactive ingredients.

Background: Biotin is a readily water-soluble substance that binds with one of the highest naturally known affinities (10⁻¹⁵ mol⁻¹) to avidin and streptavidin. The binding is almost irreversible in nature. Each of the proteins has 4 binding sites for biotin. This has an amplification effect and can be used to enhance sensitivities of immunoassays. The isoelectric point of streptavidin is in the neutral range, and streptavidin contains no carbohydrate fractions. This means that streptavidin is useful to lower non-specific binding and backgrounds caused by coating or carbohydrate-lectin interactions, in comparison to avidin.
The ortho-phenylenediamine (OPD) is a substrate which forms a water soluble yellow product. The reaction can be stopped with 1M H₂SO₄ and the product formed is orange.

Usage: This conjugate/substrate system can be used for the detection of biotin-labeled substances, eg., biotinylated antibodies, which can be used for a variety of applications. For example, ELISA for antigen quantification, and for screening procedures for detection of hybridoma antibodies. Biotinylated preparations, eg. biotinylated antibodies can be purchased, or synthesised by the user.

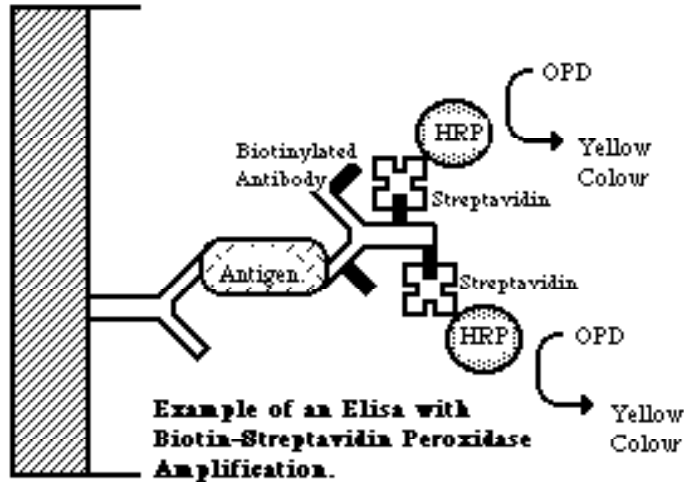
Storage: Refrigerate all components at 2-8⁰C until expiry. *Do not freeze!*

Preparation: The Amplification Reagent is supplied as ready-to-use. With some applications, a titration of the Amplification Reagent may be required. If the Amplification Reagent requires dilution, we recommend using 0.1M phosphate buffered saline (PBS). Working dilutions of the Amplification Reagent must be used within 24 hours.
To prepare the working substrate solution, place 1 OPD tablet per 5mL Substrate Buffer in a vial and wait until the tablets have completely dissolved and no bubbles remain (1-2 minutes). Place stopper on vial and mix by inversion. Substrate solution should remain clear. The working substrate solution must be used within 30 minutes of preparation.

Continues

Technical Data Sheet...continued

ELEGANCE Streptavidin-Peroxidase Development Kit



Instructions for use in ELISA:

The following protocol is recommended for use in ELISA applications. The volume of Amplification Reagent and substrate solution used should relate to the volume of antibody coated in the wells.
 Wash plate 4 times
 Add 100uL Amplification Reagent



Incubate shaking for 10 minutes at room temperature

Wash plate 4 times
 Add 100uL Substrate Solution (Stop reaction if required with 1M H₂SO₄)
 Read absorbance of plate and calculate results

Absorbance Measurements:

The OPD produces a yellow water-soluble product.
 For KINETIC assays, read at a wavelength of 435nm (or 450nm). Ensure that the reading occurs during the linear phase of the colour development.
 For ENDPOINT assays, stop the reaction when appropriate with 50uL 1M H₂SO₄. The product will be a more orange water-soluble product. Read at a wavelength of 490nm. The reading step should be carried out within 30 minutes of stopping the reaction.
When to stop the endpoint reaction: The point at which the substrate reaction is stopped is determined by the user and the ELISA reader. The OD values should be monitored and the substrate reaction should be stopped before the positive wells are no longer readable. To reduce the intensity of the reaction, it is recommended that the antibody concentration or the Amplification Reagent concentration are further diluted. Do not dilute the substrate solution further.

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Technical Data Sheet...continued

***ELEGANCE* Streptavidin-Peroxidase Development Kit**

Special Precautions: *Preservatives:* It is recommended to add preservatives to buffers used. Sodium azide inactivates the peroxidase, and so should be avoided in any step in contact with the Amplification Reagent. We recommend 0.01% thiomersal.

Contamination: Use dedicated dispensers for the enzyme/substrate reagents and use clean glassware for substrate solution preparation. Avoid contact with metallic surfaces which can interfere with substrate.

Other Products: Bioclone also has other ready-to-use ELISA products (refer to technical data sheets for information):

<i>ELEGANCE</i> TMB Substrate Solution	Ref#ETMB5	(50mL)
<i>ELEGANCE</i> ELISA Plate Blocking Buffer	Ref #EBLOCK	(1000mL)
<i>ELEGANCE</i> ELISA Wash Concentrate (15x)	Ref #EWC5	(250mL)
<i>ELEGANCE</i> Stopping Solution	Ref #ESS25	(130mL)

Precautions: For research use only. Not for human or diagnostic use. Not for therapeutic use. Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides. Avoid any skin contact with the substrate OPD. The substrate solution of OPD should be disposed of according to local regulations.

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Technical Data Sheet:

ELEGANCE TMB Substrate Solution

Reference#: ETMB5 (50mL)

Description: Substrate solution 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide for use with peroxidase in immunological detection systems. Ready-to-use.

- Contents:** *TMB Substrate Solution*
50mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a stabilising solution.
- Background:** The 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide is a substrate which forms a water-soluble blue product. The reaction can be stopped with 1M H₂SO₄ and the product formed is yellow.
- Usage:** This substrate solution can be used for the detection of peroxidase-labelled substances, eg., streptavidin-peroxidase or antibodies conjugated to peroxidase and can be used for a variety of applications. For example, ELISA for antigen quantification.
- Storage:** Refrigerate at 2-8⁰C until expiry. *Do not freeze!* Supplied as *ready-to-use*. Prior to use, bring the reagent to room temperature.
- Instructions for use in ELISA:** The following protocol is recommended for use in ELISA applications. The volume of *ELEGANCE* TMB Substrate Solution used should relate to the volume of antibody coated in the wells.
Wash plate 4 times.
Add 100µL TMB Substrate Solution (Stop reaction if required with 1M H₂SO₄).
Read absorbance of plate and calculate results.
- Absorbance Measurements:** The TMB produces a blue water-soluble product.
For KINETIC assays, read at a wavelength of 650nm. Ensure that the reading occurs during the linear phase of the colour development.
For ENDPOINT assays, stop the reaction when appropriate with 50uL 1M H₂SO₄. The product will be yellow water-soluble product. Read at a wavelength of 450nm. The reading step should be carried out within 30 minutes of stopping the reaction.
When to stop the endpoint reaction: The point at which the substrate reaction is stopped is determined by the user and the ELISA reader. The OD values should be monitored and the substrate reaction should be stopped before the positive wells are no longer readable. To reduce the intensity of the reaction, it is recommended that titrations of conjugate or initial antibody be carried out, and further diluted. Do not dilute the substrate solution further.
- Special Precautions:** *Preservatives:* It is recommended to add preservatives to buffers used. Sodium azide inactivates the peroxidase, and so avoid in any step in contact with the enzyme. We recommend 0.01% thiomersal.
Contamination: Use dedicated dispensers for the enzyme/substrate reagents and use clean glassware for substrate solution. Avoid contact with metallic surfaces which can interfere with the substrate.
- Other Products:** Bioclone also has other ready-to-use ELISA products (refer to technical data sheets for information):
- | | | |
|---|-------------|------------|
| <i>ELEGANCE</i> Streptavidin-Peroxidase Development Kit | Ref #ESDK96 | (96 wells) |
| <i>ELEGANCE</i> ELISA Plate Blocking Buffer | Ref #EBLOCK | (1000mL) |
| <i>ELEGANCE</i> ELISA Wash Concentrate (15x) | Ref #EWC5 | (250mL) |
| <i>ELEGANCE</i> Stopping Solution | Ref #ESS25 | (124mL) |
- Precautions:** For research use only. Not for human or therapeutic use.

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Technical Data Sheet:

ELEGANCE ELISA Plate Blocking Buffer

Reference#: EBLOCK (1 Litre)

Description: Buffer designed to block unbound sites on microwells after coating procedures, and to reduce non-specific binding and increase stability of coated material. Ready-to-use.

Contents: *ELISA Plate Blocking Buffer:*
1L of buffered solution containing proteins and surfactants. Contains Thiomersal, 0.01% w/v. Ready-to-use.

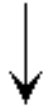
Usage: ELISA microtitre plates which have been coated with antibodies or antigens may have sites left on the plate which react with sample antigens to produce high non-specific binding. This may be reduced by treating the plate with a protein solution to block the remaining sites and to minimise non-specific binding. The *ELEGANCE* ELISA Plate Blocking Buffer contains several ingredients which combine to ensure that unwanted reactions do not occur. Note particularly that the formulation also generally imparts an increased stability to the coated materials.

Storage: Refrigerate ELISA Plate Blocking Buffer at 2-8°C until expiry.

Preparation: The blocking buffer is supplied as ready-to-use. Before use, bring the solution to room temperature.

Instructions for use in ELISA: The following protocol is recommended for use in ELISA applications. The volume of blocking buffer used should be at least double the volume of antibody or antigen coated in the wells:

Coat the plates with solution of antibody or antigen as required.
Wash plate 4 times
Add 250uL *ELEGANCE* ELISA Plate Blocking Buffer



Incubate for 2 hours at room temperature

Plates can then be:

- * stored in this buffer and washed before use
- * washed and used immediately
- * washed, dried and used within certain validated time

We recommend that the plates be stored at 4°C.

Wash plate 4 times

Run ELISA according to protocol.

Other Products: Bioclone also has other ready-to-use ELISA products (refer to technical data sheets for information):

<i>ELEGANCE</i> Streptavidin-Peroxidase Development Kit	Ref #ESDK96	(96 wells)
<i>ELEGANCE</i> TMB Substrate Solution	Ref #ETMB5	(50mL)
<i>ELEGANCE</i> ELISA Wash Concentrate (15x)	Ref #EWC5	(250mL)
<i>ELEGANCE</i> Stopping Solution	Ref #ESS25	(124mL)

Precautions: For research use only. Not for human or therapeutic use.

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Technical Data Sheet:

ELEGANCE ELISA Wash Concentrate (15X)

Reference#: EWC5 (124mL)

Description: Buffer designed to effectively wash ELISA plates and to minimise and reduce the effects of non-specific binding.

Contents: *ELISA Wash Concentrate: (15X)*
250mL of 15X Buffered wash solution. Contains Thiomersal, 0.15% w/v. To be diluted before use.

Usage: ELISA plates require effective washing to minimise effects of non-specific binding and to ensure that maximum sensitivity and precision is obtained. Plates can be washed manually or with an automatic plate washer. The *ELEGANCE* ELISA Wash Concentrate is formulated to effectively wash ELISA plates.

Storage: Refrigerate ELISA Wash Concentrate (15X) at 2-8°C until expiry.

Preparation: The ELISA Wash Concentrate should be diluted to the working wash solution by adding the desired amount of wash concentrate to a measuring cylinder and making the volume up with deionised or distilled water. Mix well before use. After dilution the wash solution can be stored at 20-25°C for up to 12 weeks.

Concentrate	Dilute to	Enough for
50 mL	750 mL	1 plate
100 mL	1.5 L	2 plates
250 mL	3.75 L	5 plates

Instructions for use in ELISA: The following protocol is recommended for use in ELISA applications. We recommend aspirating the reaction liquid and rinsing each well 4 times with 250 uL of wash solution. Avoid overflows from one well to another. After the final wash, the plate should be inverted and tapped firmly on absorbent lint-free tissue to remove the last traces of wash buffer. Ensure that no air bubbles remain in the wells before proceeding to the next step.

The wash solution should be stored in a clean container to prevent contamination with substances which could interfere. The washer should not be left standing with wash solution for long periods of time. At the end of each day, the washer should be rinsed with distilled water.

Other Products: Bioclone also has other ready-to-use ELISA products (refer to technical data sheets for information):

<i>ELEGANCE</i> Streptavidin-Peroxidase Development Kit	Ref #ESDK96	(96 wells)
<i>ELEGANCE</i> TMB Substrate Solution	Ref #ETMB5	(50mL)
<i>ELEGANCE</i> ELISA Plate Blocking Buffer	Ref #EBLOCK	(1000mL)
<i>ELEGANCE</i> Stopping Solution	Ref #ESS25	(124mL)

Precautions:
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Technical Data Sheet:

ELEGANCE Stopping Solution

Reference#: ESS25 (124mL)

Description: Stopping solution 1M H₂SO₄ for use with substrate reactions. Ready-to-use.

Contents: *ELISA Stopping Solution:*
130mL 1M H₂SO₄.

Usage: The peroxidase-TMB or peroxidase-OPD enzyme-substrate reactions can be stopped with 1M H₂SO₄ and the product formed is then stable for endpoint reading of absorbances.

Storage: Refrigerate at 2-8°C until expiry. *Do not freeze!*

Preparation: Supplied as *ready-to-use*. Prior to use, bring the reagent to room temperature.

Instructions for use in ELISA: The following protocol is recommended for use in ELISA applications. It is recommended that 50µL of Stopping Solution (1M H₂SO₄) be used. Read absorbance of plate and calculate result.

Absorbance Measurements: *OPD Chromogenic Substrate:*
For ENDPOINT assays, stop the reaction when appropriate with 50uL 1M H₂SO₄. The product will be a slightly orange water-soluble product. Read at a wavelength of 490nm. The reading step should be carried out within 30 minutes of stopping the reaction.

TMB Chromogenic Substrate:
For ENDPOINT assays, stop the reaction when appropriate with 50uL 1M H₂SO₄. The product will be a yellow water-soluble product. Read at a wavelength of 450nm. The reading step should be carried out within 30 minutes of stopping the reaction.

When to stop the endpoint reaction: The point at which the substrate reaction is stopped is determined by the user and the ELISA reader. The OD values should be monitored and the substrate reaction should be stopped before the positive wells are no longer readable. To reduce the intensity of the reaction, it is recommended that titrations of conjugate or initial antibody be carried out, and further diluted. Do not dilute the substrate solution further.

Special Precautions: *Preservatives:* It is recommended to add preservatives to buffers used. Sodium azide inactivates the peroxidase, and so avoid in any step in contact with the enzyme. We recommend 0.01% thiomersal. *Contamination:* Use dedicated dispensers for the enzyme/substrate reagents and use clean glassware for substrate solution. Avoid contact with metallic surfaces which can interfere with the substrate.

Other Products: Bioclone also has other ready-to-use ELISA products (refer to technical data sheets for information):

<i>ELEGANCE</i> Streptavidin-Peroxidase Development Kit	Ref #ESDK96	(96 wells)
<i>ELEGANCE</i> TMB Substrate Solution	Ref #ETMB5	(50mL)
<i>ELEGANCE</i> ELISA Plate Blocking Buffer	Ref #EBLOCK	(1000mL)
<i>ELEGANCE</i> ELISA Wash Concentrate (15x)	Ref #EWC5	(250mL)

Precautions:
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Specific Scientific Services

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General Activities:

The following represents core activities that can be contracted. Other operations can be discussed with Bioclone on a case-by-case basis.

- Bulk mouse ascites production *
- Preparative Mab purification
- Component formulation
- Filtration
- Bottling/ capping/ labelling
- Heat sealing, shrink wrapping
- Freeze drying *
- Microtitre plate processing
 - (confidential Bioclone process providing superior stability by ‘dry plate’ technology).
- Assay development from inception or improvement of pre-existing method *
- Bundled-in QC

[* further detail available in the following pages]



Monoclonal Antibody Production (Mouse Hybridoma Technology):

Stage 1- Immunization

- **Development protocol**

Immunization based on standard procedure. May need to change if no or low immune response or customer needs.

- **Antigen**

Amount available - Need 3-5mg.

Purity: homogeneity required for highly specific antibodies.

Assume that purified sample is provided by customer.

Size: if molecules are less than 3000-5000 daltons need to couple to carriers. Make the assumption that if small, it is supplied coupled to an immunogen.

- **Immunogenic response**

Elicit a primary antibody response (a strong secondary one if required).

- **Bleed mice weekly and do test for immune response**

(A total of 1-3 months for Stage 1)

Stage 2 - Hybridoma Production

STAGE 2A

- **Media preparation**
- **Grow myeloma cell line up to exponential phase prior to fusion**
- **Prepare feeder layer plates prior to fusion**
- **Fusion**
- **Grow cells**
- **Selection and screen for antibodies**

Purification of cells supernatant.



STAGE 2B

- **Subcloning** (for stable hybridoma cell lines)

Selection and screen for antibodies.

(No guarantees can be given other than that the monoclonal antibody recognizes the antigen supplied).

(A total of 3 months for Stage 2)

Stage 3 - Ascites Production

- **Amount required depends on customer order and of cell line / mouse yields**
- **Pristane mice**
- **Injection of hybridoma cells into mice and production of ascites fluid**
(Probably use 2-3 clones).
- **Purification if customer requires purified antibodies**

(A total of 3 months for Stage 3)



Freeze Drying (*Lyophilization*):

Introduction

- In basic terms, freeze drying is a method of drying by removing water from a product by sublimation and desorption. This is achieved by freezing and subjecting the product to high vacuum.
- Freeze drying is beneficial for heat-sensitive product such as biological materials that cannot be dried using heat (eg. Biological standards and control material can contain proteins, specific antigens and/or serum, which are destroyed by heat).
- Freeze-drying is a complex and expensive form of drying and is usually restricted to delicate, heat-sensitive materials of high value.
- **Lyophilization** is derived from the Greek “**made solvent-loving**” – due to the porous structure left after the ice has sublimed, freeze dried products rehydrates easily and quickly.

Why remove water?

- To increase stability and shelf life.
- All living organisms require water for function.
- The removal of water decreases/retards microorganism growth and enzyme activity – biological activity.

How is freeze drying achieved?

There are three stages to freeze-drying:

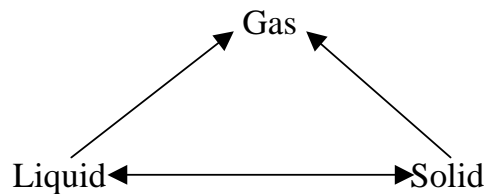
1. *Freezing:*

- Solidification of all of the water molecules in the product.
- The rate of cooling will influence the structure of the frozen matrix :
 - ◆ If the water freezes quickly, ice crystals will be small; this may cause a finer pore structure in the product and lead to a longer primary drying time.
 - ◆ If the freezing is slow, ice crystals will be larger and this can result in a product with a coarser pore structure.



2. *Primary drying:*

- The frozen products are placed in a reinforced freeze drying chamber. Pressure is reduced and heat is applied without melting the product to cause the frozen free water (unbound water) to sublime. (Sublimation is the transformation of the ice directly into water vapor without first passing through the liquid state).



- The product must be held below the eutectic temperature - the temperature at which the product is in solid state – freezing point.
- As the water sublimates, the product cools. Therefore the product will remain colder than the shelf temperature, which is supplying the heat for sublimation.
- Control rate of drying and heating at the primary stage:
 - ◆ If drying proceeds too rapidly, the dried product can be blown out of the container by escaping water vapor and lost.
 - ◆ If the product is heated too quickly, it will melt or collapse and may degrade the product and will affect the physical characteristics of the dried product, making it visually unappealing and harder to reconstitute.
- At the endpoint of primary drying, the product temperature will rise towards the shelf temperature.

3. *Secondary drying:*

- The shelf temperature is increased to desorb bound water until the residual water content falls to the range required for optimum product stability. (This occurs when maximum vacuum in the dryer is achieved).

Specific conditions required

1. The product must be solidly frozen below its eutectic point.
2. A condensing surface of low temperature must be provided.
3. The system must be capable of evacuation to low pressures in a reasonable time.
4. A controlled source of heat input to the product must be employed to drive the water from the solid state to the vapor (gaseous) state.

Conditions can be varied throughout a cycle to achieve the desired physical and chemical properties that result in a stable product.



Equipment

The freeze dryer consists of:

- a drying chamber with temperature controlled shelves
- a condenser to trap water removed from the product
- a cooling system to supply refrigerant to the shelves and condensers
- a vacuum system to reduce the pressure in the chamber and
- condenser to facilitate the drying process.

The condenser must have sufficient surface area and cooling capacity to hold all of the sublimed water from the batch at a temperature lower than the product temperature. If the temperature of the ice on the condenser is warmer than the product, water vapor will tend to move toward the product and drying will stop.

The freeze dryer is designed to create a set of conditions which maintains the optimum temperature / pressure difference for a given product to allow the transfer of moisture in an efficient time.

It should be noted that Bioclone has a BL10 Cuddon freeze dryer. (Capacity 10kg= 10L).



Immunoassay Development Overview:

Bioclone's quality management system provides a structure for immunoassay development encompassing the following phases:

Product Quality Goals and Policies

The product produced must be safe, meet the defined specifications and perform adequately over its entire shelf-life.

Risk Management

The likelihood and severity of a potential hazard is defined, evaluated, and addressed.

Design Input

Market research, regulatory requirements, and manufacturing requirements define the product's design, as it relates to product function and performance.

Design Output

Product is produced and constant review of detailed product documentation (drawings, specifications, procedures, etc.) ensures that the product conforms to the Design Input requirements.

Design Review

Formal review sessions are scheduled to assess the progress of the project.

Design Verification

An independent team verifies that the product produced meets the Design Input requirements.

Design Validation

The product is tested under intended-use conditions.

Design Changes

Any changes to the product are documented, reviewed, and approved prior to implementation.

Design History

A Design History File containing all key documentation accumulated during the product design process is maintained and reviewed to assure that the product produced conforms to the original design input.

Design Transfer

Prototype and pilot production batches are generated to ensure successful large-scale manufacture of the final product.



Project Evaluation

Bioclone provides expert advice on projects, subject to provision of full details, which are covered by a “commercial-in-confidence” agreement.

- Product identification and accessible market potential
- Design/ development potential
- Manufacturing feasibility
- Costing
- Identification of distribution channels

Bioclone's Quality Management System certified to ISO 9001, ISO 13485, GMP and CE Mark

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