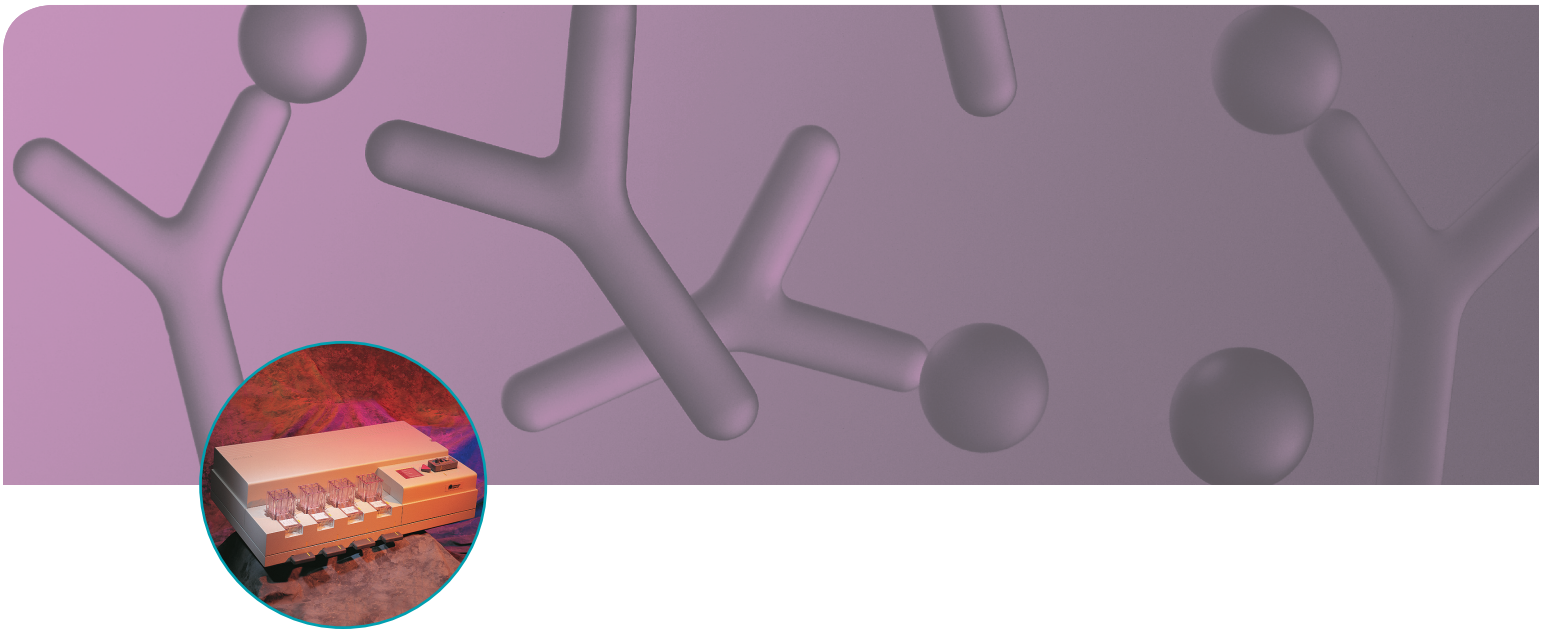
Molecular Devices

Threshold Immunoassay System

A BETTER WAY TO ANALYZE BIOMOLECULES



- USE YOUR OWN ANTIBODIES TO DEVELOP SENSITIVE IMMUNOASSAYS
- LIQUID PHASE BINDING TO MAXIMIZE MOLECULAR INTERACTION
- ACTIVE CAPTURE AND DETECTION USING A SILICON BIOSENSOR
- REDUCE SAMPLE REPLICATES
- RAPIDLY DETECT TOTAL DNA IN PICOGRAMS

The Threshold® System was developed to address difficulties scientists experience when attempting to develop highly sensitive and reproducible immunoassays without using radioactivity. While ELISAs are a common alternative, they are frequently affected by the sample matrix and often require a significant development effort to achieve uniform saturation of antibodies from well to well and plate to plate.

Further complications arise when reducing the background by blocking non-specific binding to the microplate. The results are often disappointing: the desired levels of assay sensitivity and reproducibility are not achieved. Based on these experiences, it was clear that a better way to develop sensitive immunoassays was needed. The Threshold system with Immuno-Ligand Assay (ILA) and Total DNA Assay reagents addresses this problem.

DEVELOP SENSITIVE IMMUNOASSAYS

All reagents are provided in kit form to label your antibodies (or commercially available antibodies) quickly and easily for use in the Threshold System. In a short time, you can develop a highly sensitive and reproducible assay using the ILA Detection Kit.

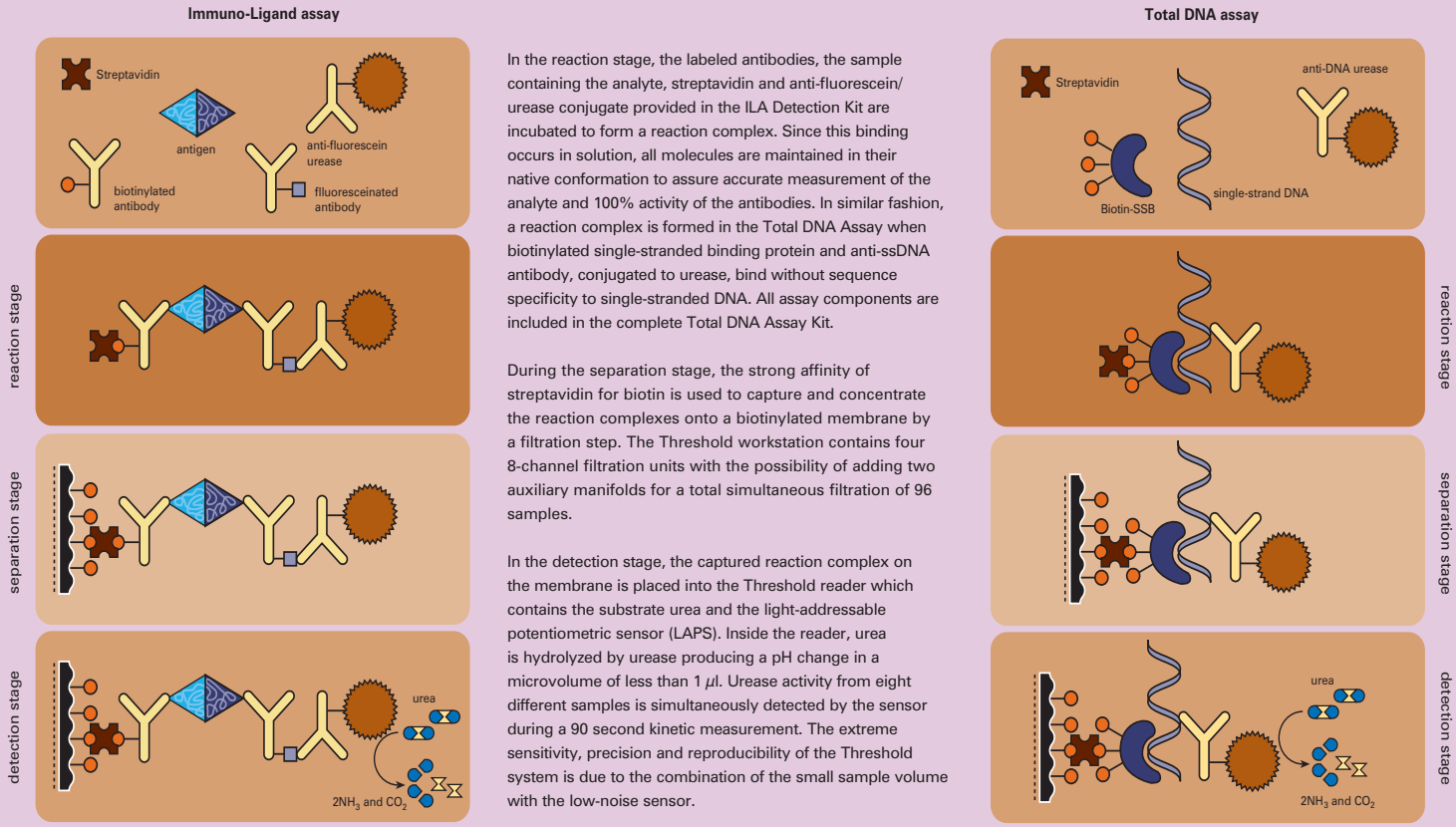
MAXIMIZE MOLECULAR INTERACTION

To optimize the interaction between the antigen and antibody, binding occurs freely in solution where the molecules are maintained in their native conformation and the antibodies retain 100% of their activity.

ACTIVE CAPTURE AND DETECTION

The antigen-antibody complex is actively captured and concentrated on a membrane using filtration and the strong affinity of streptavidin for biotin. Detection of the enzyme-labeled complex occurs by measuring the rate of enzyme turnover using a low-noise silicon biosensor. Commonly, the sensitivity is equal to or better than radioimmunoassays, and at least 10X better than ELISAs when performed with the same antibodies.

Fast and Flexible Assay Development



In the reaction stage, the labeled antibodies, the sample containing the analyte, streptavidin and anti-fluorescein/urease conjugate provided in the ILA Detection Kit are incubated to form a reaction complex. Since this binding occurs in solution, all molecules are maintained in their native conformation to assure accurate measurement of the analyte and 100% activity of the antibodies. In similar fashion, a reaction complex is formed in the Total DNA Assay when biotinylated single-stranded binding protein and anti-ssDNA antibody, conjugated to urease, bind without sequence specificity to single-stranded DNA. All assay components are included in the complete Total DNA Assay Kit.

During the separation stage, the strong affinity of streptavidin for biotin is used to capture and concentrate the reaction complexes onto a biotinylated membrane by a filtration step. The Threshold workstation contains four 8-channel filtration units with the possibility of adding two auxiliary manifolds for a total simultaneous filtration of 96 samples.

In the detection stage, the captured reaction complex on the membrane is placed into the Threshold reader which contains the substrate urea and the light-addressable potentiometric sensor (LAPS). Inside the reader, urea is hydrolyzed by urease producing a pH change in a microvolume of less than 1 µl. Urease activity from eight different samples is simultaneously detected by the sensor during a 90 second kinetic measurement. The extreme sensitivity, precision and reproducibility of the Threshold system is due to the combination of the small sample volume with the low-noise sensor.

REDUCE SAMPLE REPLICATES

Typical Threshold system assays have a dynamic range of 2 logs or greater, so fewer sample dilutions are required to accurately quantitate from the standard curve. Unmatched precision and reproducibility give you confidence in each assay.

RAPIDLY DETECT TOTAL DNA IN PICOGRAMS

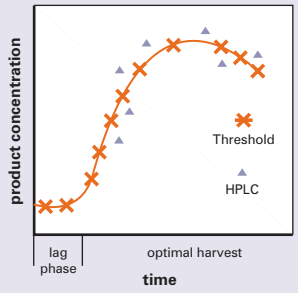
When your analyte of interest is DNA, a complete, ready-to-use kit is available which includes labeled DNA binding proteins for the sensitive detection of total DNA in biopharmaceuticals. With sensitivity as low as 2 pg, you can go from process development to final QC using the same assay.

FAST AND FLEXIBLE ASSAY DEVELOPMENT

Assays on the Threshold system may be developed using a sandwich or competitive format depending on the size and characteristics of the analyte to be detected. N-hydroxysuccinimide esters of dinitrophenyl (DNP)-biotin and fluorescein, provided in the ILA Labeling Kit, are used to reproducibly label the antibodies in about two hours. Because both haptens are chromogenic, simple photometric measurements are used to determine the number incorporated. Threshold analyzes a multitude of biomolecules:

- Proteins
- Host cell proteins
- Antibodies
- Protein a
- Protein g
- Insulin
- BSA
- Transferrin
- Toxins
- Receptor ligands
- Peptides
- Viruses
- Acetylcholinesterase
- Tick anticoagulant protein
- Nucleic acids
- Total DNA
- Specific sequence DNA
- PCR amplified DNA

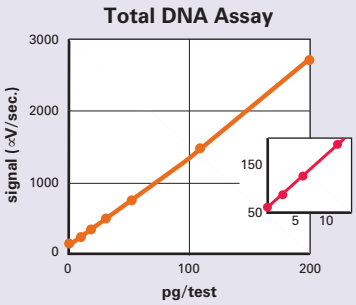
From Research to Product Approval in the Shortest Time



Fermentation Monitoring
Maximize product yield by determining optional harvest time

- → Detect onset of protein expression
- → Monitor product degradation
- → May detect conformational changes
- → Fast results in less than an hour
- → More sensitive than HPLC

The speed and sensitivity of the Threshold System allows monitoring of the early stages of protein production during fermentation. Since it is an immunoassay, protein degradation and confirmational changes may be detected by the Threshold system assay.



Quality Control
A validatable method for accurately and reproducibly quantitating contaminant levels in the final product without radioactivity

- → Precision and reproducibility typically < 10%
- → Validatable assays with quality controlled reagents and software
- → Transferable between operators
- → Replaces radioimmunoassays

The standard curve for the Threshold Total DNA Assay ranges from 2 pg to 200 pg per test, with precision and reproducibility typically less than 10%.

Assay Development
Rapidly develop sensitive immunoassays using your antibodies

- → Reduce assay development time
- → Flexible assay format—sandwich or competitive
- → Liquid phase binding
- → Less antibody per test than ELISAs
- → Wide dynamic range

E. Coli Host Cell Protein Assay

STANDARD CURVE:	2 TO 160 NG/ML
INTRA-DAY PRECISION:	3.0% CV (N=6)
INTER-DAY PRECISION:	8.4% CV (N=69)
LIMIT OF QUANTITATION:	4 PPM (4 NG HOST CELL PROTEINS/MG PRODUCT)

The liquid phase binding, reproducibility and sensitivity of the Threshold Immuno-Ligand Assay has allowed the rapid development and validation of a rugged process specific host cell protein assay.

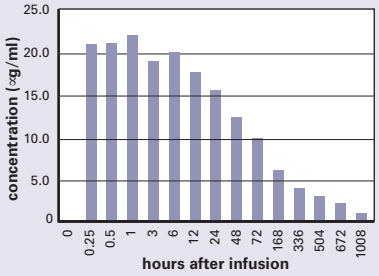
	PROTEIN LOAD (mg/ml)	PRODUCT RECOVERED	DNA (pg/DOSE)
ORIGINAL BUFFER	5	63%	18
CONDITIONS	14	81%	60,000
	16	98%	300,000
NEW CONDITIONS	5	98%	< 2

The Threshold system has been used to optimize the buffer conditions used during chromatography to maximize product yield and contaminant removal. With the original buffer, it was shown that increasing the protein load increased the product yield. However, the Threshold data showed the column was increasingly ineffective at DNA removal, as protein load increased, under the original conditions. A new protocol was developed that provided excellent product yield and DNA clearance.

Process Development
Improve media selection and optimize purification conditions

- → Rapid sample analysis streamlines development
- → Increased assay sensitivity allows dilution to minimize sample interference
- → Wide dynamic range: one assay method from purification to QC
- → No radioactivity or harmful chemicals

Pharmacokinetics of Human IgG_1 Monoclonal Antibodies to *Pseudomonas aeruginosa* Type a Flagellae in Phase I Patient Sera by Dr. William Landsperger, Baxter Healthcare Corporation

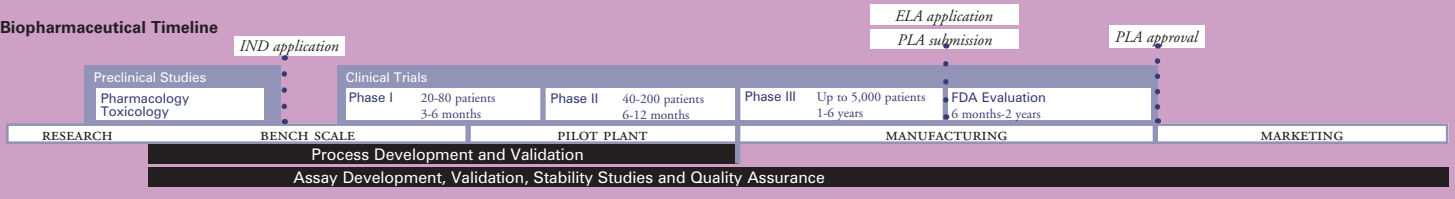


Clinical Assays
Evaluate drug clearance and immune response in clinical samples

- → High sensitivity – equal to or better than RIA
- → Perform pharmacokinetic studies
- → Evaluate immunogenicity

The Threshold system has been used to monitor the concentration of a product antibody in human serum over a 6 week period with reproducibility and sensitivity as low as 1.2 µg/ml in serum.

Biopharmaceutical Timeline



DNA CONTAMINATION TESTING

The following is a list of recombinant proteins tested for DNA contamination by the Threshold Total DNA Assay:

- Anti-inflammatory agents
- Atriopeptide
- CD 4
- Colony stimulating factors
- Erythropoietin
- Factor VIII
- Fibroblast growth factor
- Follicle stimulating hormone
- GP 120
- Hemoglobin
- Hirudin
- Human growth hormone
- Human serum albumin
- Immunotoxins
- Insulin
- Insulin-like growth factor
- Insulinotropin
- Interferons
- Interleukins
- Monoclonal Ab
- Neuropeptides
- Somatotropins
- Superoxide dismutase
- Tissue plasminogen activator
- Urokinase
- Vaccines
- Viral antigens

THRESHOLD SOFTWARE

Threshold Software not only controls the Threshold Workstation, but also collects and analyzes data. It includes five standard curve fits to provide the highest accuracy when quantitating unknowns. A complete summary report detailing the results can be printed or exported for further analysis.

The optional Threshold Enterprise Software has a Spike Recovery Report for analysis of the results within the software application, a complete validation package and security tools that allow current and future Threshold users to comply with GLP/GMP/FDA 21 CFR Part 11 requirements. Threshold Enterprise includes the following security tools:

- Individual passwords and user IDs
- Permission-dependent access to major features
- Audit trail of log-on and log-off activities and all user actions that modify the file
- Signed electronic statements

REAGENTS

The Threshold immunoassay system has several reagents available:

- Total DNA Assay Kit
- ILA Detection Kit
- ILA Labeling Kit

ORDERING INFORMATION

Threshold Immunoassay System
Part Number: 0200-0500

- (1) Threshold workstation
- (1) Pentium®-based computer w/monitor
- (1) Color inkjet printer
- (1) THS software for Windows® 95/ NT
- (2) Cleanable reader/electrode kits
- (1) ILA or DNA Starter Kit

Total DNA Assay Kit (8 sticks):
Part Number: R9009

Total DNA Assay Kit (Bulk):
Part Number: R9004

ILA Detection Kit:
Part Number: R9003

ILA Labeling Kit:
Part Number: R9002

Threshold Enterprise Software:
Part Number: 0200-6059

Enterprise Administrator™ Software:
Part Number: 0200-6014

Threshold Enterprise Validation Package:
Part Number: 0200-6064

SALES OFFICES

United States and Canada
Molecular Devices
Tel. +1-800-635-5577
Fax +1-408-747-3601

Brazil
Molecular Devices Brazil
Tel. +55-11-3616-6607
Fax +55-11-3616-6607

China
Molecular Devices Beijing
Tel. +86-10-6410-8669
Fax +86-10-6410-8601

Molecular Devices Shanghai
Tel. +86-21-6887-8820
Fax +86-21-6887-8890

Germany
Molecular Devices GmbH
Tel. +49-89/96-05-88-0
Fax +49-89/9-62-02-34-5

Japan
Molecular Devices Japan, Osaka
Tel. +81-6-6399-8211
Fax +81-6-6399-8212

Molecular Devices Japan, Tokyo
Tel. +81-3-5282-5261
Fax +81-3-5282-5262

South Korea
Molecular Devices Korea, LLC
Tel. +82-2-3471-9531
Fax +82-2-3471-9532

United Kingdom
Molecular Devices Ltd.
Tel. +44-118-944-8000
Fax +44-118-944-8001

www.moleculardevices.com

6/10 #0120-1331B