

A gloved hand holding a vial with yellow liquid. The background is a blurred laboratory setting with various equipment and containers.

Building Effective Drugs of Abuse Assays: Conjugates, Kinetics, and Customization

Medix Biochemica

Before we get started

 Please use the Q&A button at the bottom of your screen to submit any questions.

There will be a Q&A session at the end of this webinar. If we do not have time to answer your questions, we will reach out by email following the webinar.

 This webinar is being recorded. The recording as well as on-demand link will be released following the webinar.

Independent, International, and Industry-Leading Raw Materials Supplier

- Provider of high-quality antibodies, antigens, proteins, enzymes, biospecimens
- Experts in immunoassays, clinical chemistry, molecular diagnostics
- Our portfolio is among the most comprehensive in the IVD industry
- Enabling our customers to develop and manufacture quality IVD tests



Featured Speakers and Panelists



Laura Kolsi, PhD

Technology Manager
Medix Biochemica



Maria Voutilainen, PhD

Global Product Manager
Medix Biochemica



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R&D Scientist
Medix Biochemica



Ella-Maria Vesilahti, MSc

R&D Scientist
Medix Biochemica





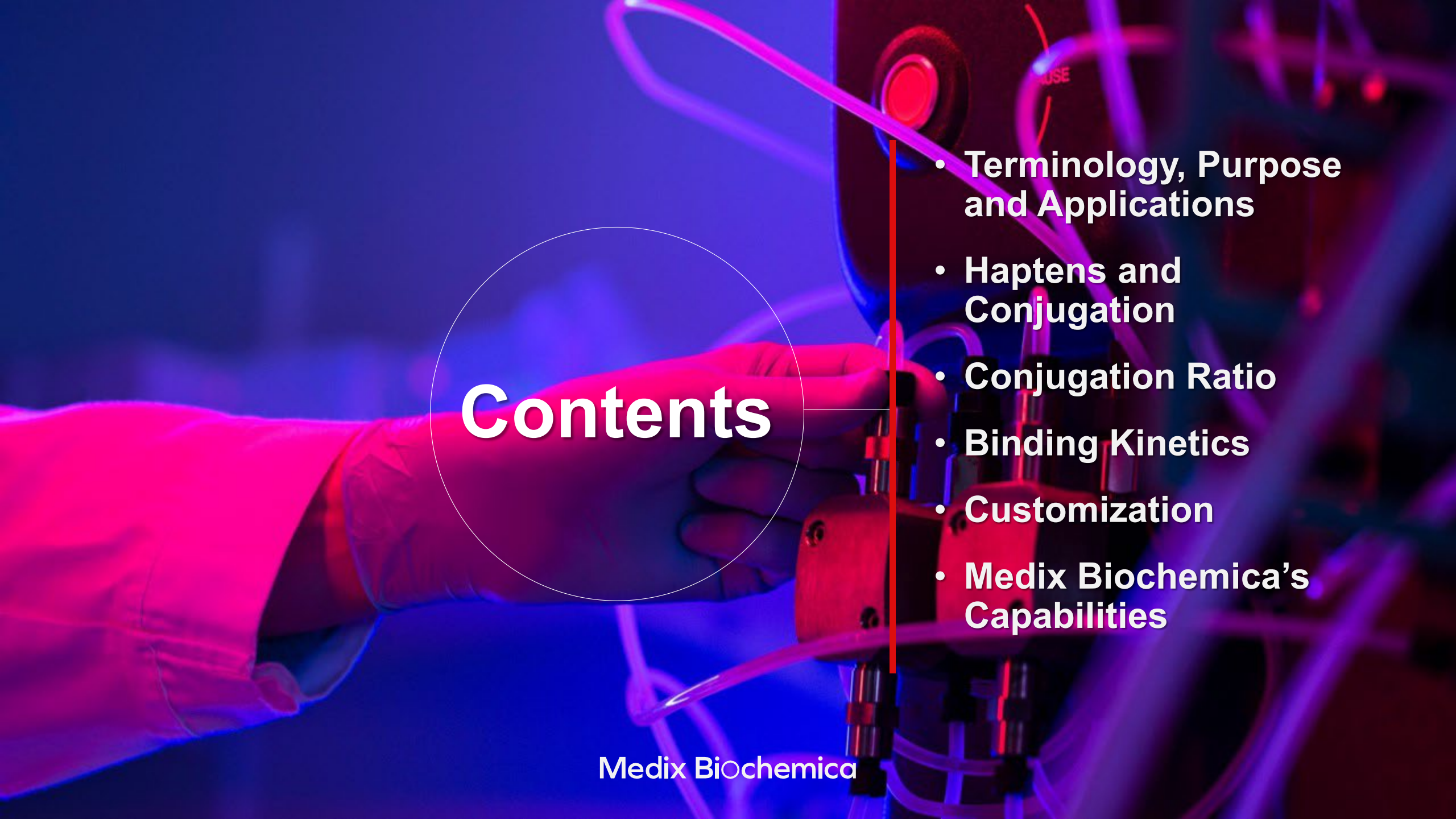
Building Effective Drugs of Abuse Assays: Conjugates, Kinetics, and Customization

Laura Kolsi, PhD

Technology Manager

30th September 2025

Medix Biochemica



Contents

- Terminology, Purpose and Applications
- Haptens and Conjugation
- Conjugation Ratio
- Binding Kinetics
- Customization
- Medix Biochemica's Capabilities

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01

Terminology, Purpose and Applications

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Terminology and Definitions

- **DoA** = Drugs of Abuse, **illegal substances** and **prescription drugs** used improperly
- **DoA Antigen Conjugate** = **hapten** + **protein/label** – together can trigger an **immune response**
- **Hapten** = **small molecule** that **mimics** the structure of the **analyte** and binds to **antibody**
- **Protein/label** = Carrier molecule to which hapten is chemically attached

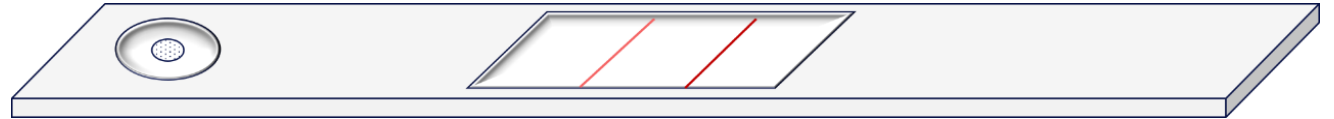


Image: [Photo](#) by Unknown Author is licensed under [CC BY-SA](#)

Applications – Role of DoA Antigen Conjugates

- Purpose
 - Used in immunoassays to detect specific analytes, especially small molecules like drugs of abuse
- Common assay formats - Competitive assays

- Lateral flow
- ELISA
- EMIT



- Small molecules conjugated to enzymes (ELISA, EMIT)

ELISA

- Horseradish peroxidase (HRP) (ELISA)
- Signal (color change) is usually inversely proportional to analyte concentration

EMIT

- G6PDH
- Enzyme activity is directly proportional to the analyte concentration



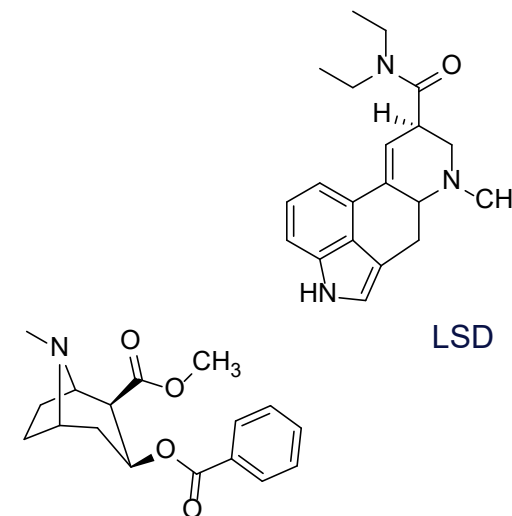
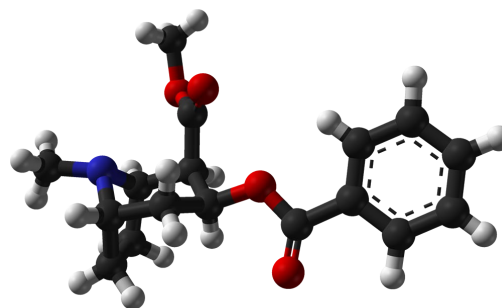
02

Haptens and Conjugation

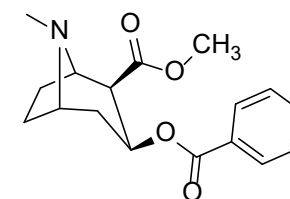
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Haptens

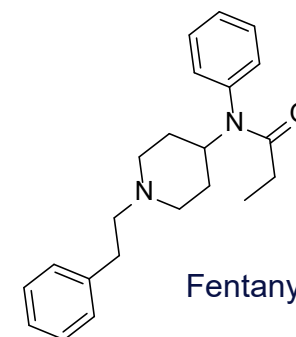
- Small molecules that include a linker for the protein conjugation
- Small molecules
 - $M_w < 1000$ Da
 - Organic compounds
 - Chemically synthesized or natural products such as alkaloids (morphine, nicotine, cocaine) or hormones
- Designing hapten structure
 - Mimics the structure of the analyte
 - Optimal binding to the antibody
 - Linker structure, length and conjugation site affects binding



LSD



Cocaine



Fentanyl

Conjugation

- Hapten is chemically conjugated to the carrier protein/label
 - Typical hapten functional groups taking part in the conjugation: COOH, SH, SCN
 - Conjugation protocols: EDC/NHS or TSTU/DIEA coupling, maleimide conjugation
- Carrier proteins include BSA, HRP, KLH, G6PDH, OVA, BTG
- Lysine and cysteine residues of the protein react with the activated hapten

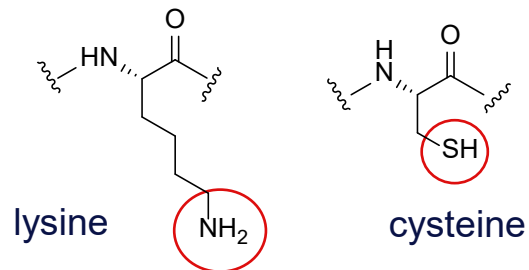
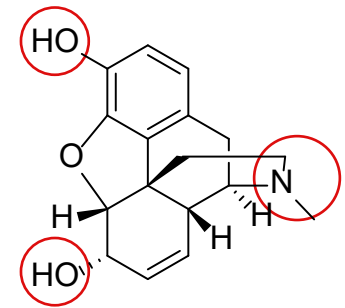


Image: Crystal structure of BSA
Source: [RCSB Protein Data Bank](#)

Example of BSA Conjugation of Morphine



Morphine = MOR

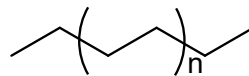
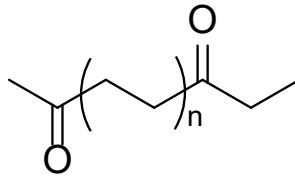
Several
synthesis
steps



MOR-R-COOH

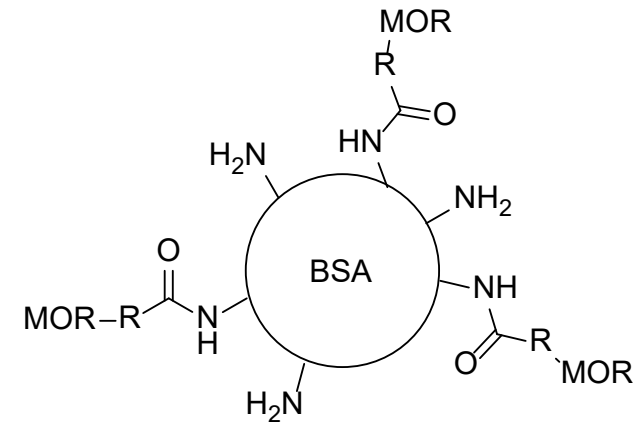
Hapten

R =



Linkers

Conjugation



Morphine BSA conjugate



03

Conjugation Ratio

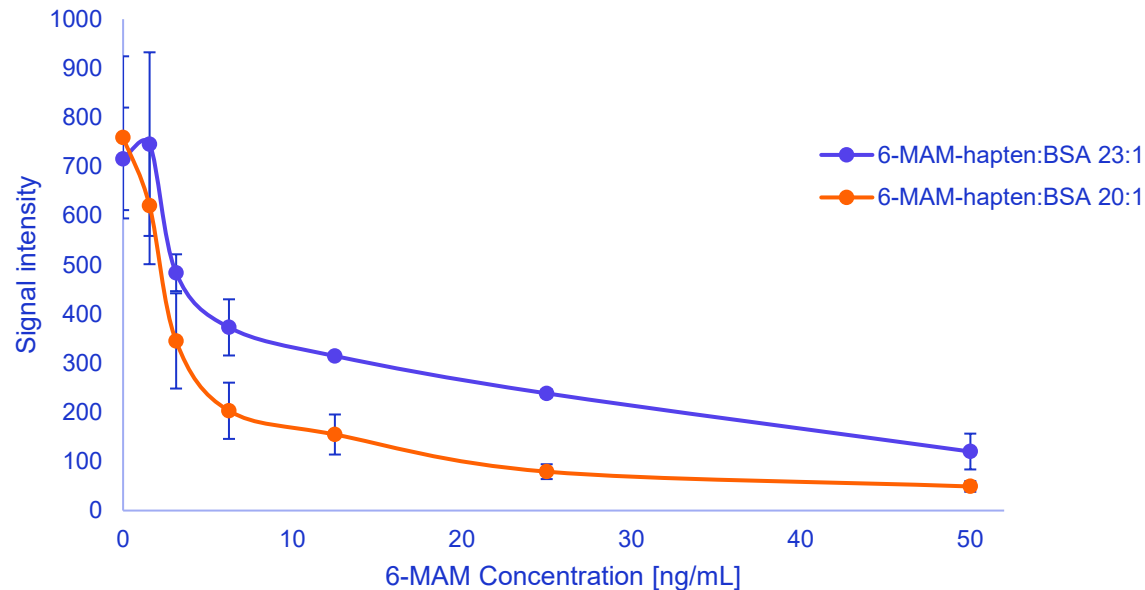
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Conjugation Ratio

- Conjugation ratio = number of molecules (haptens) attached to protein
- Can have a significant effect on assay performance (eg. sensitivity)
- Finding optimal level important (low vs. high)
 - Risk of overlabeling
 - Steric hindrance
 - Antibody detection
 - Solubility
- Enables monitoring of batch-to batch consistency

Effect of Conjugation Ratio to Assay Sensitivity – 6-MAM-BSA

6-MAM conjugate → lower conjugate ratio resulted in greater sensitivity

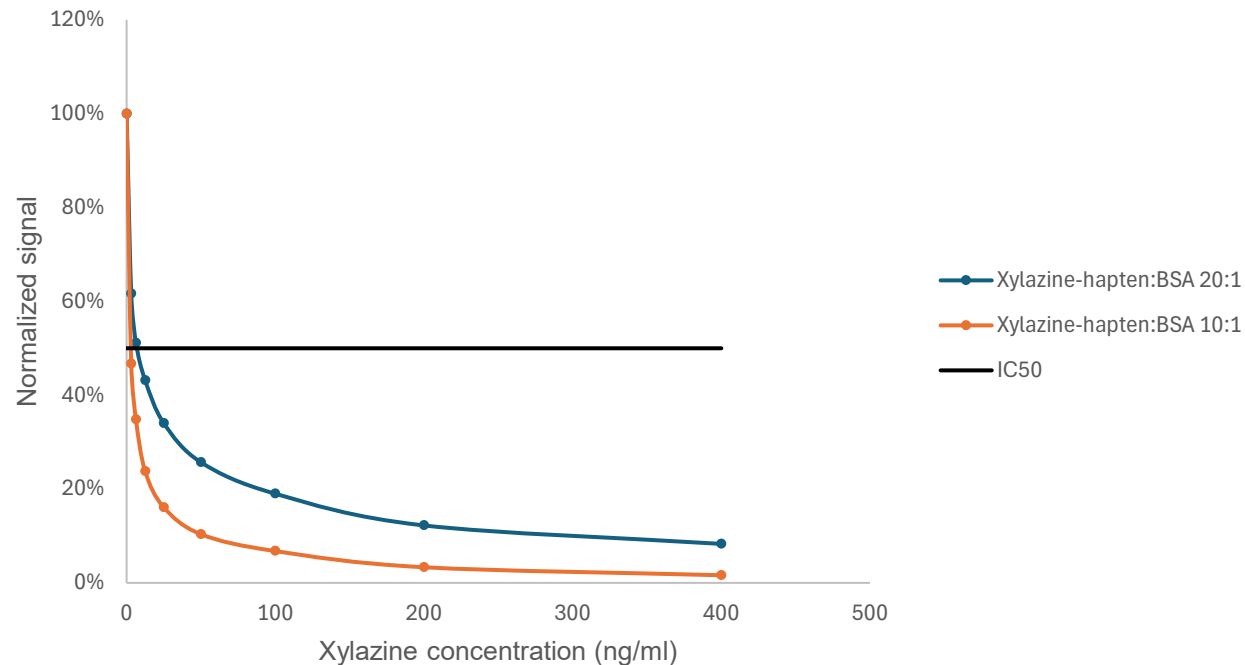


Quantified signal intensities in Lateral flow

Figure: Part of poster 'Performance Evaluation of Antibody and Antigen Conjugate Pair for Detection of Heroin Metabolite (6-MAM) in Lateral Flow Assay', medixbiochemica.com

Effect of Conjugation Ratio to Assay Sensitivity – Xylazine

- Greater sensitivity with lower conjugation ratio IC50: 7 ng/ml → 3 ng/ml

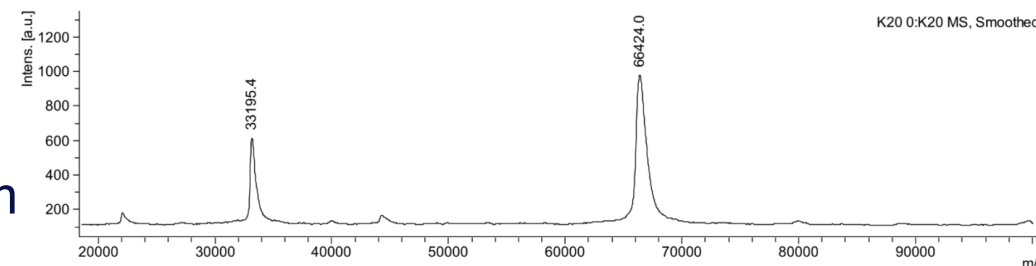


Competitive ELISA test for Xylazine

Methods for Determining the Conjugation Ratio

- Maldi-TOF MS

- Direct measurement
- Analyzing the mass differences in the mass spectrum



- TNBSA

- Detection of primary amines using 2,4,6-trinitrobenzene sulfonic acid
- Colorimetric assay
- Quantification via spectrophotometer (335-345 nm)

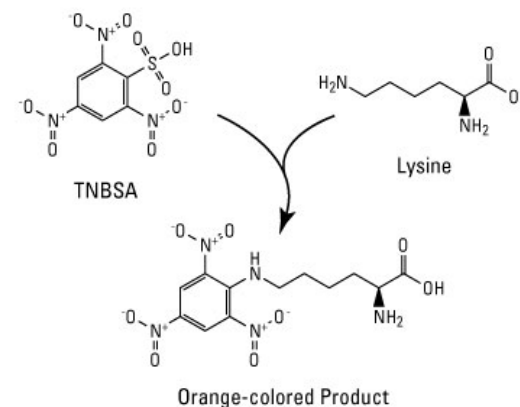


Image: [TNBSA reaction scheme for detection of primary amines | Thermo Fisher Scientific](#)

Binding Kinetics

The background image shows a laboratory environment. A multi-tiered rack holds numerous microcentrifuge tubes. Some tubes are empty, while others contain liquids of various colors (yellow, red, blue). The scene is illuminated with a mix of blue and red light, creating a high-tech, scientific atmosphere. The rack is positioned in the foreground, with other laboratory equipment visible in the blurred background.

04

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Binding Kinetics

- Affinity is determined by the equilibrium dissociation constant (K_D), which is the ratio of dissociation (k_{off}) to association (k_{on}).
- Kinetics are especially important with small molecules as they are commonly used in competitive assay set-ups
 - For example, in lateral flow, fast association rate is an advantage
- At Medix Biochemica, we report kinetic constants measured by our R&D in the product specification sheet

100998 Anti-Fentanyl 13101 SPTN-5

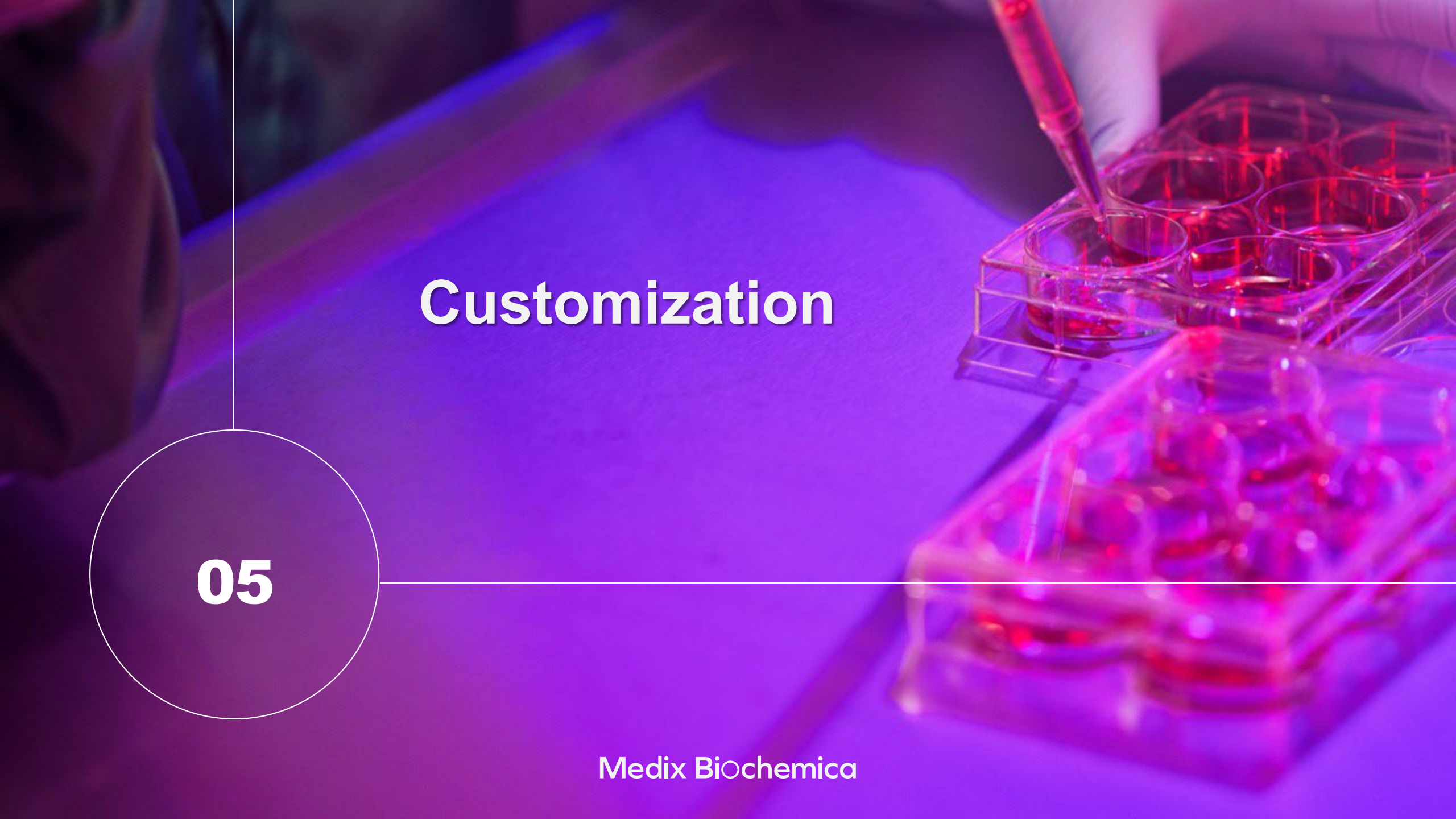
Kinetic parameters

Association rate constant	2.2×10^6 1/Ms
Dissociation rate constant	1.2×10^{-5} 1/s
Affinity constant	$K_A = 1.8 \times 10^{11}$ 1/M; $K_D = 5.3 \times 10^{-12}$ M (= 0.005 nM)
Determination method	BLI (Octet RED96e)
Determination antigen	Fentanyl-BSA Antigen, Medix Biochemica, 170040

Binding Kinetics

- Conjugation site and ratio may affect the binding kinetics with the antibody
 - Sterical reasons
 - Orientation of the hapten
- When developing a competitive assay, it is important to optimize binding kinetics between antigen conjugate and antibody

Pair	K_a (K_{on}) 1/Ms	K_d (K_{off}) 1/s	K_A 1/M	K_D M
Clone 1 + Xylazine-hapten:BSA 10:1	2.6×10^5	Does not dissociate in conditions used.		
Clone 1 + Xylazine-hapten:BSA 20:1	7.4×10^5	1.7×10^{-5}	4.4×10^{10}	1.6×10^{-11}

A laboratory setting with a blue background. In the foreground, there are two racks of test tubes containing a red liquid. A hand is using a pipette to add more red liquid to one of the test tubes. The scene is dimly lit, with the primary light source being the blue background and the red liquid.

Customization

05

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Customization

Why customize

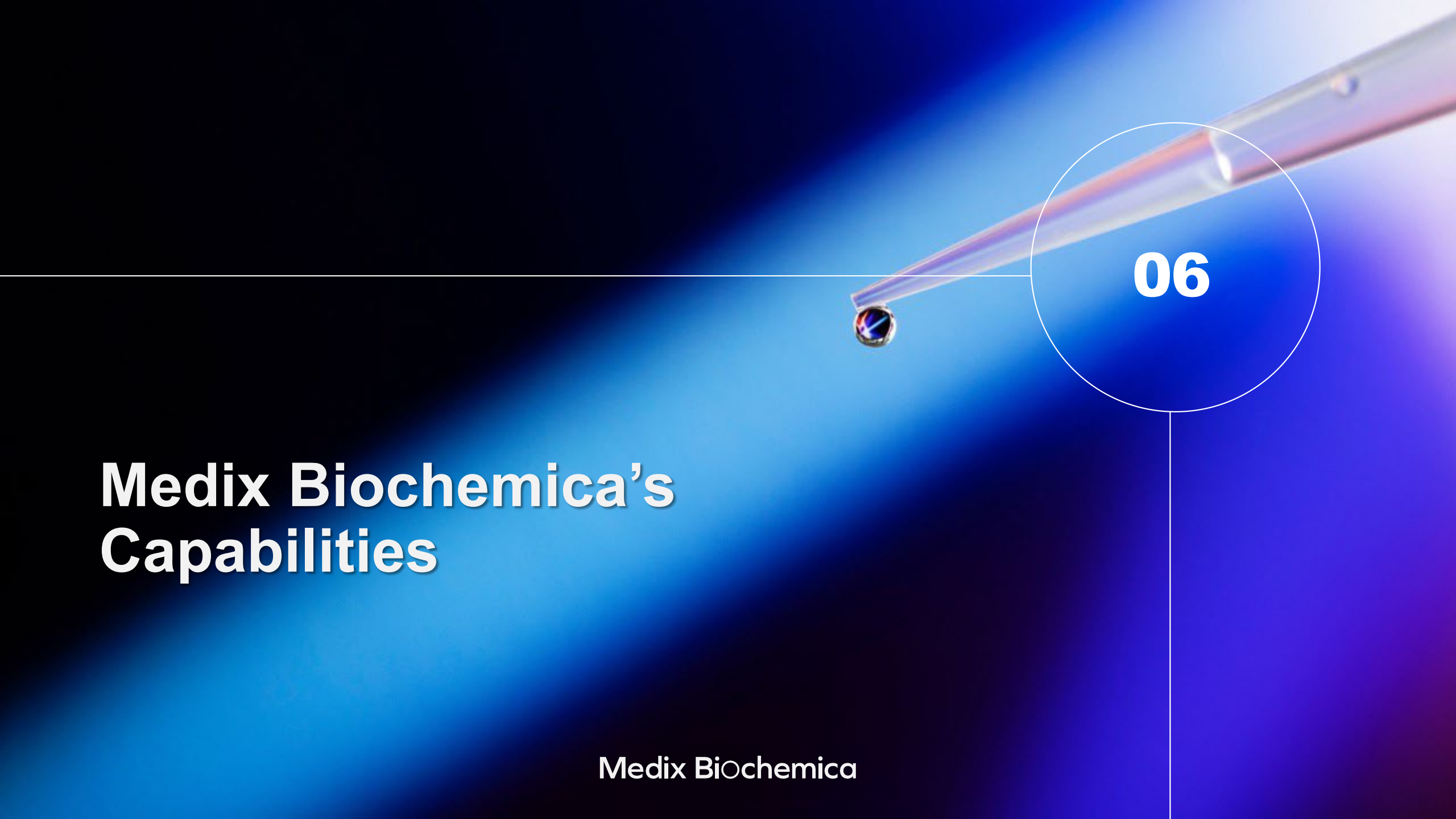
Off-the-shelf conjugates may not meet specific assay requirements

- Matrix effects
- Cross-reactivity (specificity)
- Sensitivity
- Signal intensity and lower the limit of detection

Strategies

- Choice of carrier protein (e.g. BSA, OVA, KLH, HRP)
- Engineering of the protein structure for optimal amount of conjugation sites (eg. Lysines)
- Linker chemistry and hapten orientation (linker length, conjugation site)
- Tuning the conjugation ratio



A blue gradient background with a white line and a circular graphic containing the number 06.

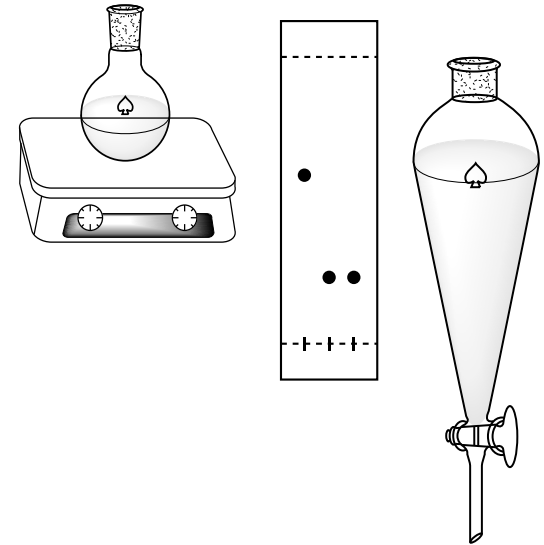
06

Medix Biochemica's Capabilities

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Medix Biochemica's Capabilities – In-house R&D and Production

- In-house
 - Hapten design: Tailored to mimic the target molecule's structure for optimal binding
 - Synthesis: Performed in-house to ensure purity and consistency
 - Conjugation – Controlled processes for optimal performance
 - Final DoA antigen conjugates available in several grams
 - QC: Constant monitoring of product quality
- ISO13485 certified manufacturing



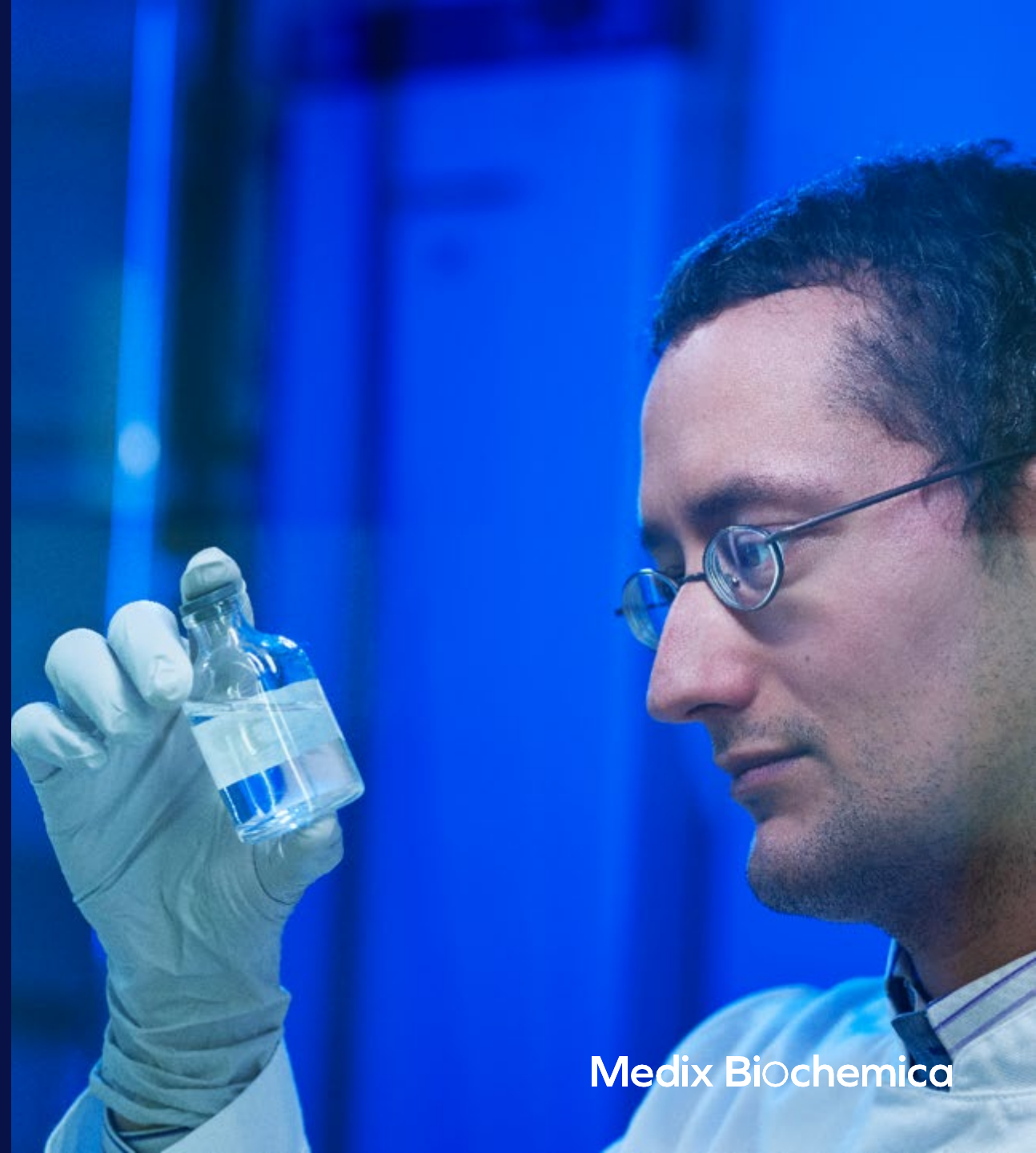
Benefits of in-house production: better control, high quality and seamless integration with antibody development

Medix Biochemica's Capabilities – Customization

Custom project opportunities for
optimal assay performance

- Changing hapten to protein ratio
(low/high)
- Tuning conjugation parameters
- Using different carrier proteins

*We are open for all opportunities
– contact us to discuss more*



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Thank you

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Building Effective Drugs of Abuse Assays: Conjugates, Kinetics and Customization

Maria Voutilainen, PhD

Global Product Manager for Drugs of Abuse

30.9.2025

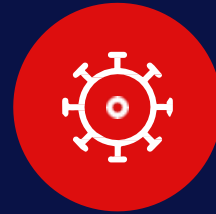
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The Qualified Supplier to the IVD Industry

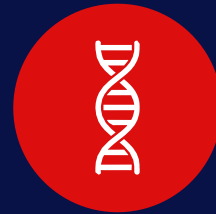
IVD test manufacturers across
the globe trust Medix Biochemica
as their partner of choice for IVD
raw materials



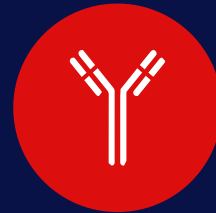
Quality



Supply Reliability

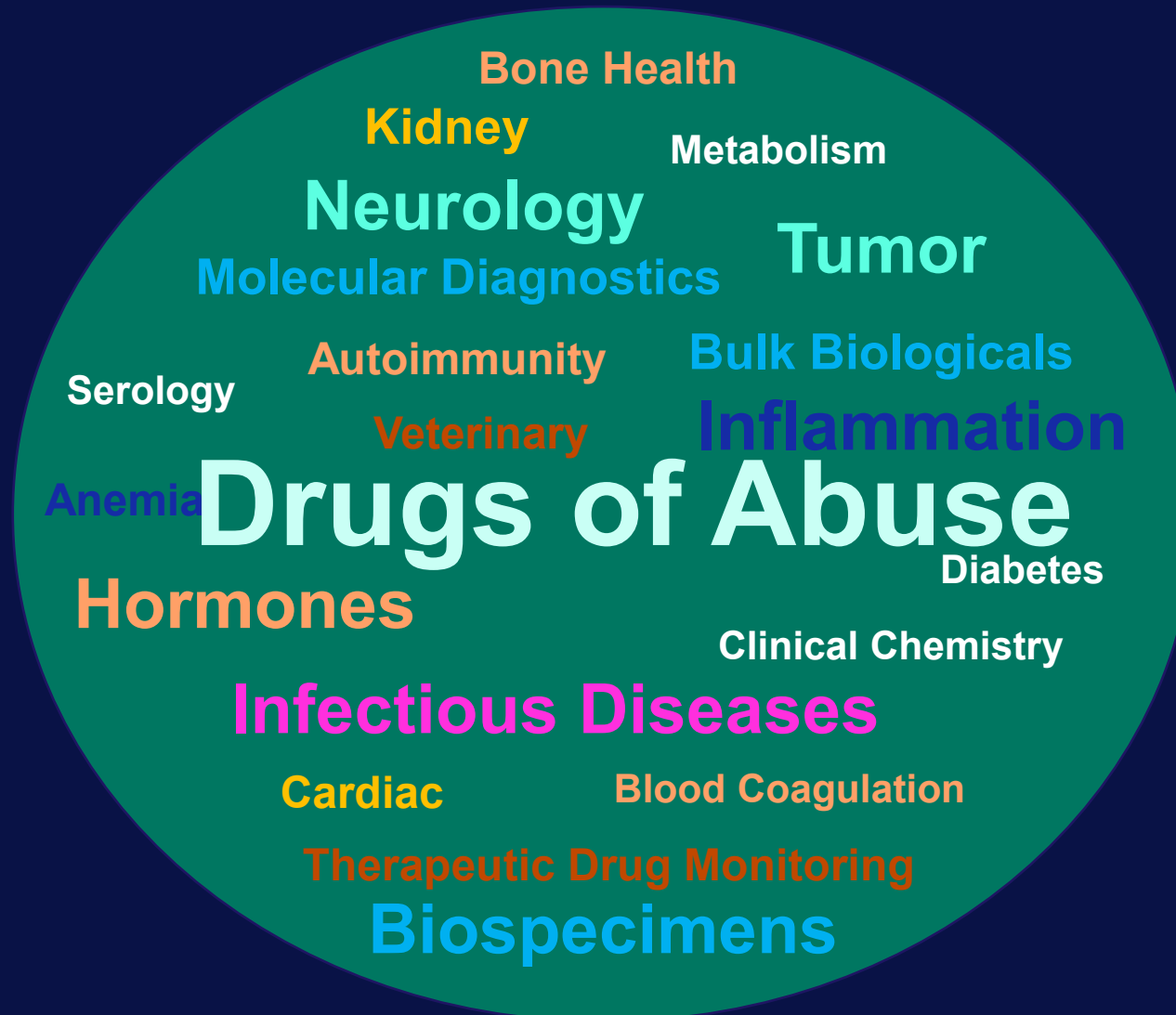


Scientific Innovation

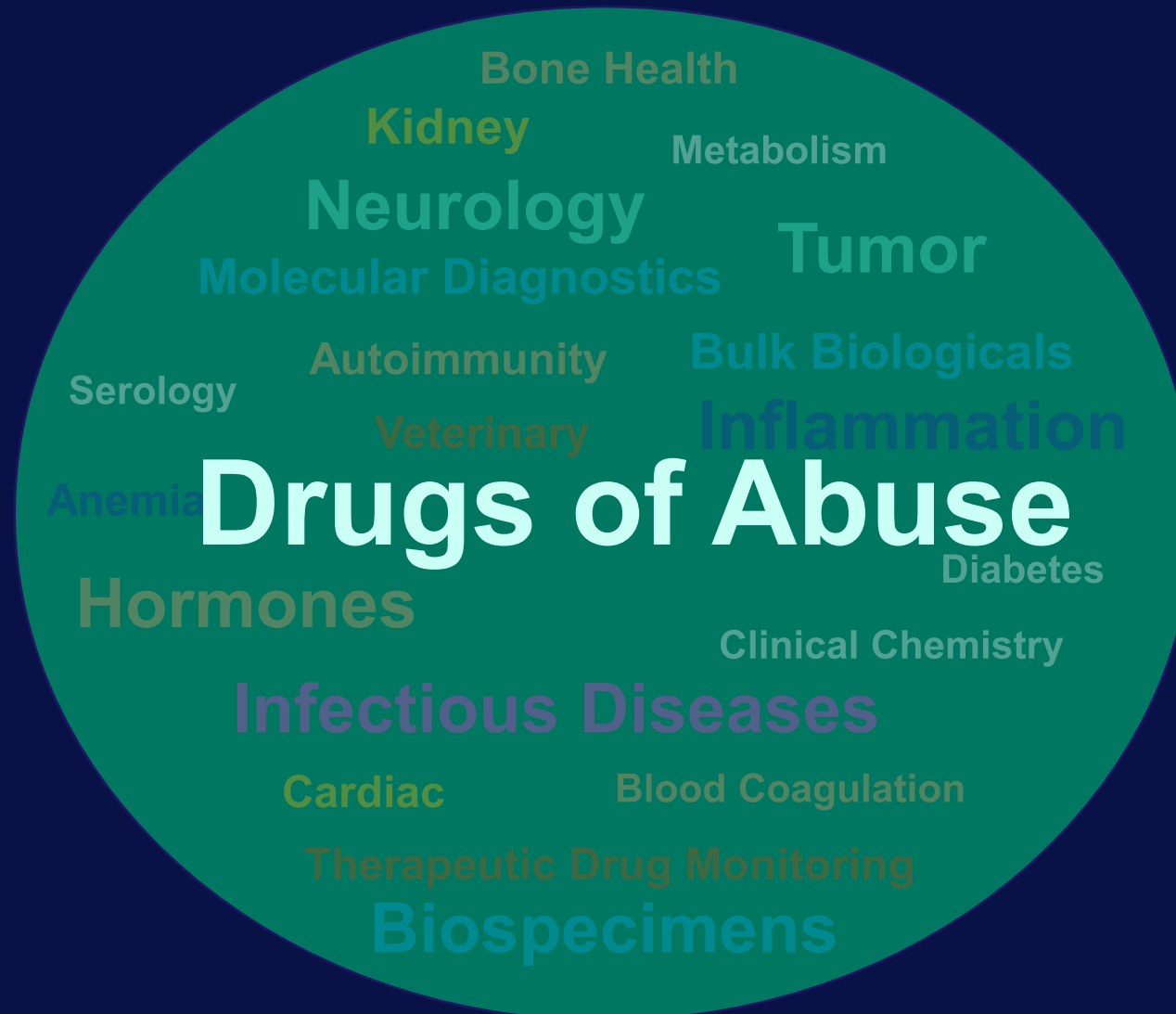


Comprehensive Portfolio

Clinical Areas Supported



Clinical Areas Supported



Medix Biochemica - DoA Portfolio

Antibodies & Antigens Conjugates

Opioids / Opiates

Hallucinogens

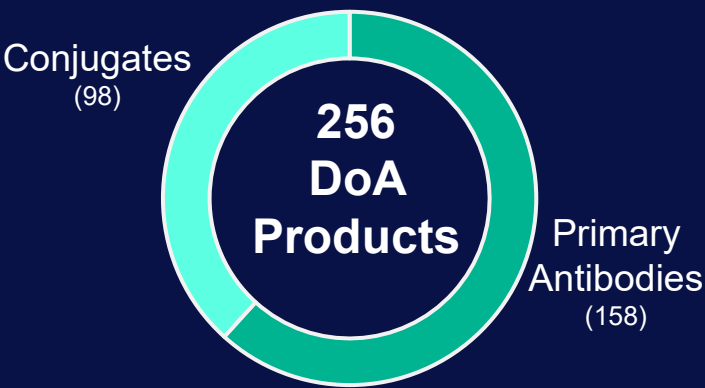
Cannabinoids /
Synthetic Cannabinoids

Depressants / Sedatives
/ Hypnotics

Stimulants

Prescription Psychiatric
Drugs /TDM

Other Psychoactives



Drugs of Abuse (DoA) Portfolio Introduction

Opioids / Opiates

- 6-Monoacetylmorphine (6-MAM)
- Buprenorphine (BUP)
- EDDP (Methadone Metabolite)
- Fentanyl
- Hydrocodone
- Methadone
- Morphine
- Oxycodone
- Propoxyphene (PPX)
- Tramadol

Stimulants

- Amphetamine
- Benzoylecgonine (Cocaine)
- MDMA
- MDPV
- Methcathinone
- Methamphetamine
- Methylphenidate
- Methylone
- Mephedrone
- Caffeine
- Nicotine
- Cotinine (Nicotine metabolite)

Other Psychoactives

- Kratom (Mitragynine)
- Salbutamol

Prescription Psychiatric Drugs /TDM

- Aripiprazole
- Clozapine
- Olanzapine
- Quetiapine
- Risperidone
- Tricyclic Antidepressants (TCA)
- Carbamazepine
- Phenytoin (Dilantin)

Cannabinoids / Synthetic Cannabinoids

- Cannabinoids (THC)
- K2
- UR-144
- Pinaca

Depressants / Sedatives / Hypnotics

- Barbiturate
- Oxazepam
- Clonazepam
- Methaqualone
- Zolpidem
- Zopiclone
- Carisoprodol
- Diphenhydramine
- Xylazine

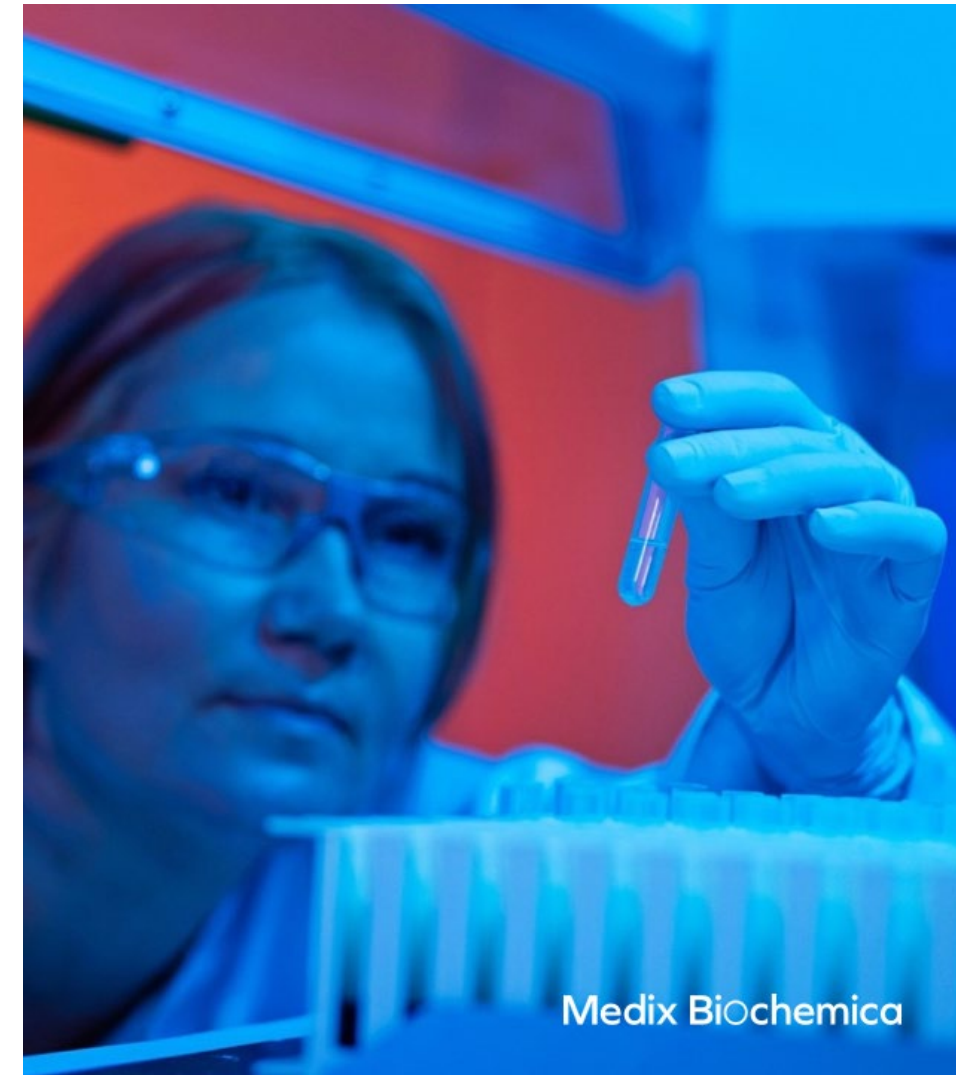
Hallucinogens

- LSD
- Ketamine
- PCP

Antibody and Antigen Conjugate Availability

Drugs of Abuse	Ab	Ag
6-MAM	■	■
Acetaminophen	■	■
Amphetamine	■	■
Barbiturate	■	■
Benzodiazepine	■	■
Buprenorphine (BUP)	■	■
Caffeine	■	■
Cannabinoids (THC)	■	■
Carisoprodol	■	■
Clonazepam	■	■
Cocaine Metabolite (Benzoylecgonine)	■	■
Cotinine	■	■
Ethanol (EtG)	■	■
Fentanyl	■	■
Hydrocodone	■	■
K2	■	■
Ketamine	■	■
Kratom (Mitragynine)	■	■
LSD	■	■
MDMA (Ecstasy)	■	■
MDPV	■	■
Methadone	■	■
Methadone metabolites (EDDP)	■	■
Methamphetamine	■	■
Methaqualone	■	■
Methcathinone	■	■

Drugs of Abuse	Ab	Ag
Methylone	■	■
Methylphenidate	■	■
Morphine	■	■
Nicotine	■	■
Oxazepam	■	■
Oxycodone	■	■
Phencyclidine	■	■
Pinaca	■	■
Propoxyphene (PPX)	■	■
Tramadol	■	■
UR-144	■	■



6-Monoacetylmorphine (6-MAM)

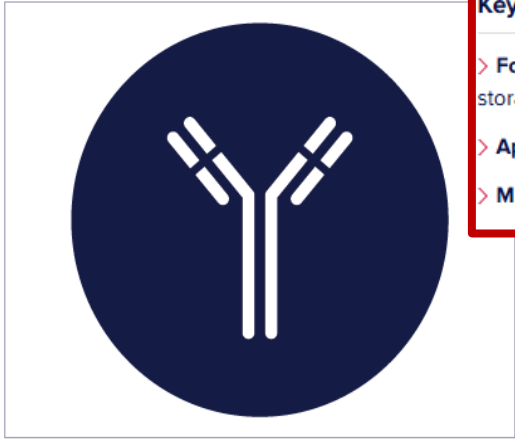
6-monoacetylmorphine (6-MAM):

Unique metabolite of heroin, and its presence confirms recent heroin use.

Product Type		Catalog #	Description
Monoclonal antibody	Y	100983	Anti-6-MAM 12705
		140041	Anti-6-MAM R12703
		HM445	6-Monoacetylmorphine (6-MAM) antibody
		HM446	6-Monoacetylmorphine (6-MAM) antibody
		HM461	6-Monoacetylmorphine (6-MAM) antibody
Polyclonal antibody	Y	HM842	6-Monoacetylmorphine (6-MAM) antibody
		JP091	6-Monoacetylmorphine (6-MAM) antibody
		JP092	6-Monoacetylmorphine (6-MAM) antibody
Antigen conjugates	+	JP093	6-Monoacetylmorphine (6-MAM) antibody
		170031	6-Monoacetylmorphine (6-MAM)-BSA antigen

Anti-6-MAM R12703 SPTN-5

Catalog Number: 140041



Key Features

- > **Form** : Liquid, may turn slightly opaque during storage
- > **Application** : FIA, Lateral Flow
- > **Matched Pairs** : 6-MAM-BSA Antigen 170031

Range of Fentanyl Antibodies and Conjugates

Fentanyl:

Synthetic opioid with 50–100 times the potency of morphine.

Product Type		Catalog #	Description
Monoclonal antibody		100974	Anti-Fentanyl 13102 SPTN-5
		100998	Anti-Fentanyl 13101 SPTN-5
		100999	Anti-Fentanyl 13103 SPTN-5
		HM004	Fentanyl antibody
		HM1132	Fentanyl antibody
		HM1133	Fentanyl antibody
Polyclonal antibody		HM628	Fentanyl antibody
		P01-99-53R-IF	Fentanyl antibody
		P01-99-54R-IF	Fentanyl antibody
		P01-99-55R-IF	Fentanyl antibody
Antigen conjugate		170040	Fentanyl-BSA antigen
		LA008	Fentanyl-BSA antigen
		LA395	Fentanyl-BSA antigen
		LA540	Fentanyl-BSA antigen
		LA556	Fentanyl-BSA antigen

- Fentanyl-specific antibodies allow selective detection of the parent drug.
- Cross-reactive antibodies provide combined recognition of fentanyl and nor fentanyl, increasing the likelihood of detection in screening assays.
- Availability of different antibody types supports assay designs that prioritize either specificity or broader sensitivity.

Range of Fentanyl Antibodies and Conjugates

Fentanyl:

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Product Type		Catalog #	Description
Monoclonal antibody		100974	Anti-Fentanyl 13102 SPTN-5
		100998	Anti-Fentanyl 13101 SPTN-5
		100999	Anti-Fentanyl 13103 SPTN-5
		HM004	Fentanyl antibody
		HM1132	Fentanyl antibody
		HM1133	Fentanyl antibody
		HM628	Fentanyl antibody
Polyclonal antibody		P01-99-53R-IF	Fentanyl antibody
		P01-99-54R-IF	Fentanyl antibody
		P01-99-55R-IF	Fentanyl antibody
Antigen conjugate		170040	Fentanyl-BSA antigen
		LA008	Fentanyl-BSA antigen
		LA395	Fentanyl-BSA antigen
		LA540	Fentanyl-BSA antigen
		LA556	Fentanyl-BSA antigen

- Fentanyl-specific antibodies allow selective detection of the parent drug.
- Cross-reactive antibodies provide combined recognition of fentanyl and nor fentanyl, increasing the likelihood of detection in screening assays.
- Availability of different antibody types supports assay designs that prioritize either specificity or broader sensitivity.

NEW Launch: Nitazenes

Available in October 2025

Key Considerations

- **Highly potent opioids**
Several analogues far more potent than fentanyl
- **Rapidly emerging class**
New structural variants frequently appear in illicit markets
- **Challenging detection**
Not yet included in standard opioid screens; may be missed, delaying diagnosis and treatment
- **Clinical presentation**
Profound respiratory depression, sedation, and high risk of fatal overdose
- **Public health impact**
Increasing prevalence globally; linked to clusters of severe intoxications and deaths

Medix Biochemica

Biologicals, Biospecimens, & Base Matrices

Drugs of Abuse
Capabilities

Common Matrix Types	
Urine	991-03
Saliva	991-05
Hair	991-14
Serum	991-24
Sweat	991-20
Meconium	991-16

Analyte Testing (Positive & Negative Options)	
Amphetamines	Methadone
Barbiturates	Mitragynine
Buprenorphine	Naloxone
Benzodiazepines	Naltrexone
Cocaine/Benzoyllecgonine	Opiates
Ethyl Glucuronide (EtG)	Tricyclic Antidepressants (TCA)
Fentanyl	THC
Gabapentin	
Drug Free (Self-Declared)	Drug Free (Certified)

Why Choose Medix Biochemica Biologicals?	
Urine	High donor counts and testing options support projects with needs ranging from a single positive sample to bulk drug free matrices
Saliva	Established protocols and recallable donors allow for high volume access with adaptable collection methods
Sweat	Single donor samples to bulk volumes with optional testing - all collected under controlled conditions
Hair	Recallable donors allow for time-course studies and the option for sample pairing with other matrices
Meconium	Availability of a difficult to source neonatal specimen that is collected in accordance with regulatory guidelines
Serum	Either positive or negative , samples available with high donor counts and pre-screened testing
General	Customization for any collection (whether demographic or process) along with the ability to offer paired matrices

Exclusive Offer of Free DoA Samples

Exclusive Offer: Free Samples

We are offering free samples of our selected DoA antibodies and antigen conjugates for a limited time.

Test our products and provide feedback!

Antibody
6-MAM
Acetaminophen
Benzoyllecgonine (Cocaine)
Buprenorphine (BUP)
Cannabinoids (THC)
Clonazepam
Ethanol (EtG)
Fentanyl
Ketamine
Kratom (Mitragynine)
LSD
MDMA
Methadone
Morphine
Oxycodone
PCP
Pinaca
Propoxyphene (PPX)
Tramadol
Tricyclic Antidepressant (TCA)
UR-144
Xylazine

Antigen Conjugate
6-MAM
Amphetamine
Benzoyllecgonine (Cocaine)
Cannabinoids (THC)
Carisoprodol
Clonazepam
Cotinine
Fentanyl
Hydrocodone
K2
Ketamine
MDMA
Methaqualone
Methylphenidate
Morphine
PCP
Propoxyphene (PPX)
Tramadol
Tricyclic Antidepressant (TCA)
Zolpidem

Not seeing your analyte of interest?
Let us know which ones and we'll be in touch on what we can offer

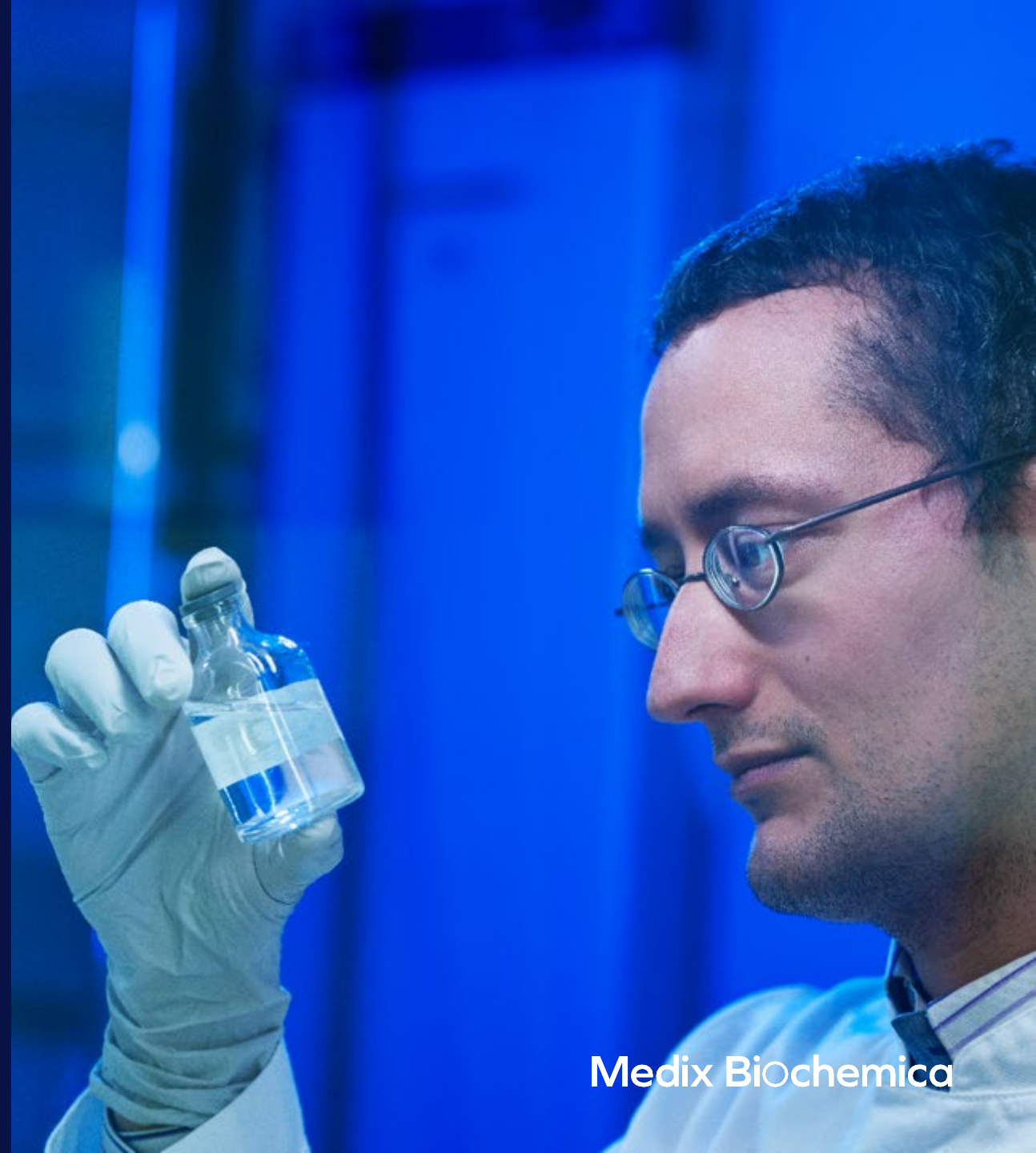


Thank you

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