Immunoassays for Process-related Impurities

Immunoassays for the detection of process-related impurities in biologics

Creative Diagnostics provides highly sensitive and specific ELISA Kits for monitoring process-related impurities in biologics

CD Creative Diagnostics®

Content

Background

Biopharmaceuticals are a class of drugs generally produced in living organisms that can be used for the treatment of a wide variety of diseases. Biopharmaceutical samples include hormones, enzymes, monoclonal antibodies, vaccines, and blood factors, and each presents different challenges during drug development and manufacturing, one of which is the presence of process-related residual impurities and their levels in the final biopharmaceutical drug product.

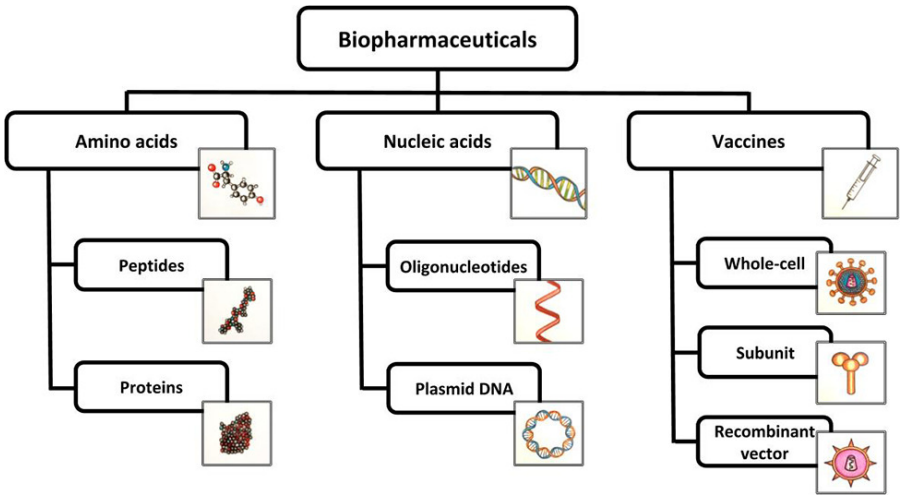


Fig. 1 The classification of biopharmaceuticals into three main categories, namely, amino acids, nucleic acids, and vaccines. (Rasmussen, A. S. B., *et al*. 2021)

Process-related impurities are basically any chemicals or biological entities expected to be present in a manufacturing process that is either not completely removed during purification or not desired to be in the API (Active pharmaceutical ingredient) or the final drug product. Process-related impurities can be divided into three categories:

- Cell substrate-derived impurities (e.g., proteins/nucleic acids derived from the host organism, such as host cellular DNA).
- Cell culture-derived impurities (e.g., inducers, antibiotics, serum and other media components).
- Downstream-derived impurities (e.g., enzymes, chemical and biochemical processing reagents—cyanogen bromide, reducing agents, inorganic salts, solvents etc.).

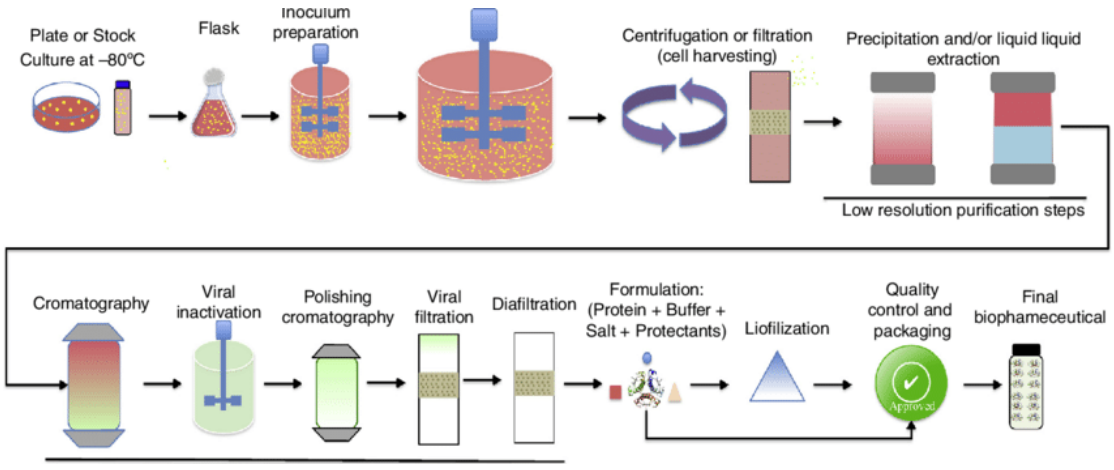


Fig. 2 The biopharmaceutical manufacturing technology flowchart exemplifying the upstream and downstream bioprocess. (Jozala, A. F., *et al*. 2016)

Effective removal of process-related impurities or process residues is very important for pharmaceutical and biopharmaceutical development. To meet the requirements for monitoring and controlling process-related impurities and contaminants in the biological manufacturing process as described in the ICH Q6B Biomolecule Production Guidelines, Creative Diagnostics has developed a series of highly sensitive ELISA kits to analyze impurity residues. These kits are suitable as good impurity detection tools used in biomanufacturing processes.

Cell substrate-derived impurities

During the manufacturing of biopharmaceuticals, some non-product, host cell-derived materials are inevitably introduced into the process stream. The major process-related safety concerns are residual host cellular DNA and residual host cell proteins (HCPs), which must be removed by purification processes.

Host cellular DNA

Since host cellular DNA may contain oncogenic or tumorigenic sequences, both in terms of quantity and size, in the genetically engineered viruses, it is important to ensure that such process-related impurities are kept to a minimum. While several methods are available to measure very low levels of residual host cellular DNA (e.g, DNA probe hybridization, Threshold® single stranded DNA binding analysis), quantitative polymerase chain reaction (qPCR), also known as real-time PCR, has become the most widely used method.

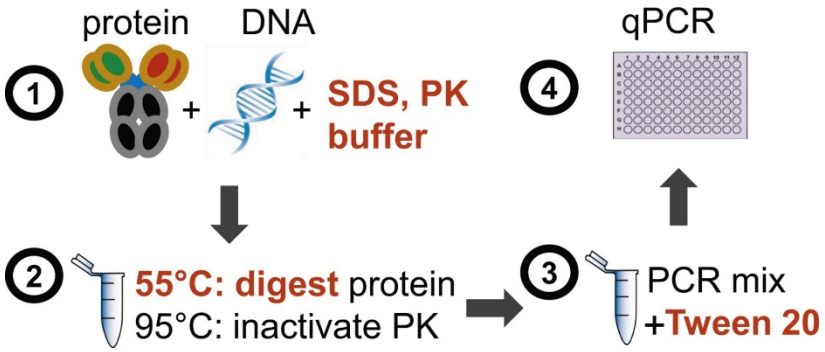


Fig. Flowchart of the direct qPCR approach. (Peper, G., *et al*. 2014)

Ralated Product

Cat. No	Product Name / Service	Sensitivity
DEIA-BY050	CHO DNA residue ELISA Kit	3 ppt
DEIA-BY051	*E. coli* DNA residue ELISA Kit	0.03 ppb
DEIA-BY052	Human DNA residue ELISA Kit	0.03 ppb
DEIA-BY053	Human residual DNA fragment Assay Kit	0.03 ppb
HCD-S001	ppb-level quantitative detection -- Fluorescence staining / Threshold Assay / qPCR Assay	-

Host cell proteins

The primary safety concern with HCPs in biopharmaceuticals is their potential to induce anti-HCP antibodies that could lead to clinical effects in patients. In addition, HCPs may act as adjuvants and induce anti-drug antibodies that may affect the safety or efficacy of the drug. HCPs can also have a direct effect on the quality of the product itself. For example, proteolytic HCPs, even in minute amounts, can cleave the desired recombinant protein or monoclonal antibody over time, reducing or eliminating biological potency or altering stability. While several methods are available to measure trace amounts of residual HCPs (e.g., two-dimensional HPLC, mass spectrometry), HCP immunoassays (typically the enzyme-linked immunosorbent assay, ELISA) remain the most widely used method.

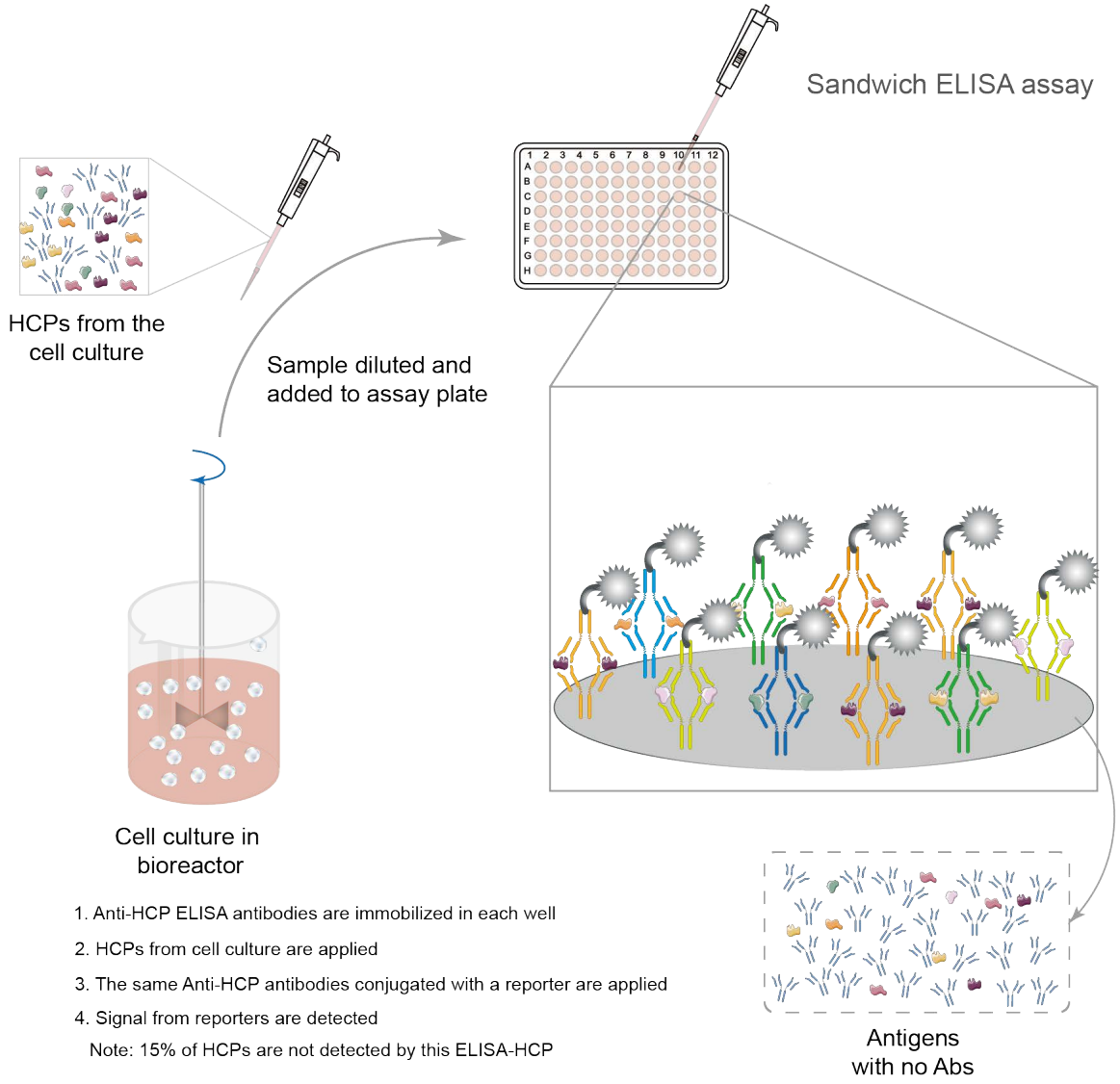


Fig. A typical sandwich ELISA for host cell proteins

Ralated Product

Host	Cat. No	Product Name
Mammalian	DEIABL475	A549 HCP ELISA Kit
	DEIABL476	BHK HCP ELISA Kit
	DEIABL478	CHO Host Cell Protein ELISA Kit
	DEIABL482	HEK 293 HCP ELISA Kit
	DEIABL484	HeLa HCP ELISA Kit
	DEIABL486	MDCK HCP ELISA Kit
	DEIABL487	MRC5 HCP ELISA Kit
	DEIABL488	NS/0 HCP ELISA Kit
	DEIABL490	PER.C6 HCP ELISA Kit
	DEIABL496	SP2/0 Host Cell Protein ELISA Kit
	DEIABL498	Vero Cell Host Cell Proteins
	DEIASL149	HEK 293T HCP ELISA Kit
	DEIA-BY012	CAP Cell Line HCP ELISA Kit
Yeast	DEIABL491	Pichia pastoris HCP ELISA Kit
	DEIABL494	S. cerevisiae HCP ELISA Kit
Bacterial	DEIABL481	*E. coli* HCP ELISA Kit
	DEIABL485	L. lactis HCP ELISA Kit
	DEIABL489	P. fluorescens HCP ELISA Kit
	DEIABL493	S. aureus HCP ELISA Kit
Insect	DEIABL495	SF9 HCP ELISA Kit

Cell culture-derived impurities

The cell culture medium provides the complex mixture necessary for cell survival and biopharmaceutical production. Different culture media are composed of amino acids, vitamins, sugars, and inorganic salts. However, cell culture media may also include serum (e.g., fetal bovine serum), growth factors (e.g., insulin- like growth factor), selective agents (e.g., methotrexate), antibiotics, and process-enhancing

agents.

Culture Media Components

Serum typically contains albumin, immunoglobulins, and a rich mixture of other proteins. When these components are present as residual impurities, immunogenicity should also be considered. For example, for cell culture produced vaccines, extraneous proteins known to be capable of causing allergenic effects in humans should not be added to a final virus medium of cell culture-derived vaccines intended for injection.

Insulin and insulin-like growth factor-1 (IGF-1) are widely used growth factors to delay apoptosis in mammalian cell culture. However, growth factor residuals in biopharmaceuticals may become a concern for affecting the drug activity.

Ralated Product

Analyte	Cat. No	Product Name	Sensitivity
Insulin	DEIA-BY021	Insulin ELISA Kit	0.125 ppb
Albumin	DEIA9947	Human ALB(Albumin) ELISA Kit	0.5 ppb
	DEIA605	Bovine ALB ELISA Kit	0.69 ppb
	DEIASL274	Rabbit anti-BSA IgG ELISA Kit	1:200
	DEIASL275	Human anti-BSA Antibody ELISA Kit	1:200
Transferrin	DEIA1523	Transferrin Human ELISA Kit	1.5 ppb
	DEIA7585	Bovine Transferrin ELISA Kit	0.2 ppb
Species-Specific Immunoglobulin	DEIA611	Bovine IgA ELISA Kit	1.37 ppb
	DEIA612	Bovine IgG ELISA Kit	3.439 ppb
	DEIA613	Bovine IgG1 ELISA Kit	1 ppb
	DEIA614	Bovine IgG2 ELISA Kit	1 ppb
	DEIA615	Bovine IgM ELISA Kit	1.37 ppb
	DEIASL056	Camel IgG ELISA Kit	1.17 ppb
	DEIA-BY033	Cat IgG ELISA Kit	0.8 ppb
	DEIA-BY034	Cat IgM ELISA Kit	3.1 ppb
	DEIA627	Chicken IgA ELISA Kit	15.63 ppb
	DEIA3658	Chicken IgG (IgY) ELISA Kit	1.56 ppb

DEIA629	Chicken IgM ELISA Kit	1.56 ppb
DEIA-BY035	Cow IgA ELISA Kit	3.91 ppb
DEIA-BY036	Cow IgG ELISA Kit	0.78 ppb
DEIA-BY037	Cow IgM ELISA Kit	15.63 ppb
DEIA7614	Canine IgA ELISA Kit	0.02 ppb
DEIA7616	Canine IgG ELISA Kit	0.5 ppb
DEIA7617	Canine IgM ELISA Kit	0.2 ppb
DEIA631	Dog IgA ELISA Kit	3.91 ppb
DEIA632	Dog IgE ELISA Set	0.78 ppb
DEIA633	Dog IgG ELISA Kit	2.5 ppb
DEIA688	Human IgA ELISA Kit	1.875 ppb
DEIA9467	Human IgE ELISA Kit	6.25 ppb
DEIA1480U	Human IgG ELISA Kit	0.78 ppb
DEIA5459	Human IgG1 ELISA Kit	0.94 ppb
DEIA691	Human IgM ELISA Kit	0.94 ppb
DEIA7713	Horse IgA ELISA Kit	7.81 ppb
DEIA7715	Horse IgG ELISA Kit	3.13 ppb
DEIA647	Horse IgM ELISA Set	15.63 ppb
DEIA640	Goat IgG ELISA Kit	7.81 ppb
DEIA-BY038	Goat IgM ELISA Kit	12.5ppb
DEIA7756	Guinea pig IgG ELISA Kit	3.13 ppb
DEIA-BY039	Guinea Pig IgM ELISA Kit	3.91 ppb
DEIABL406	Monkey IgA ELISA Kit	3.91 ppb
DEIABL407	Monkey IgG ELISA Kit	3.91 ppb
DEIABL408	Monkey IgM ELISA Kit	3.91 ppb
DEIA-BY041	Monkey IgE ELISA Kit	5 ppb

DEIA-BY042	Monkey IgG1 ELISA Kit	6.25 ppb
DEIA651	Mouse IgA ELISA Kit	0.93 ppb
DEIA652	Mouse IgE ELISA Kit	1.56 ppb
DEIA8704	Mouse IgG ELISA Kit	7.81 ppb
DEIA6402	Mouse IgG1 ELISA Kit	1.56 ppb
DEIA655	Mouse IgG2a ELISA Kit	0.195 ppb
DEIABL411	Mouse IgG2b ELISA Kit	0.195 ppb
DEIA658	Mouse IgG3 ELISA Kit	0.78 ppb
DEIA6405	Mouse IgM ELISA Kit	7.81 ppb
DEIA663	Pig IgA ELISA Kit	4.69 ppb
DEIA664	Pig IgG2b ELISA Kit	1.37 ppb
DEIA665	Pig IgM ELISA Kit	15.6 ppb
DEIA-BY054	Pig IgG ELISA Kit	1.56 ppb
DEIA-T6101	Porcine IgG ELISA Kit	0.5 ppm
DEIA8497	Rabbit IgA ELISA Kit	0.281 ppb
DEIA8498	Rabbit IgG ELISA Kit	3.13 ppb
DEIA8499	Rabbit IgM ELISA Kit	7.81 ppb
DEIA674	Rat IgA ELISA Kit	2.34 ppb
DEIA675	Rat IgE ELISA Kit	1.56 ppb
DEIA676	Rat IgG ELISA Kit	1.95 ppb
DEIA681	Rat IgM ELISA Kit	1.95 ppb
DEIA-BY043	Rat IgG1 ELISA Kit	15.63 ppb
DEIA-BY044	Rat IgG2a ELISA Kit	7.81 ppb
DEIA-BY045	Rat IgG2b ELISA Kit	1.58 ppb
DEIABL420	Sheep IgG ELISA Kit	15.63 ppb
DEIA-BY046	Sheep IgA ELISA Kit	0.156 ppm

	DEIA-BY047	Sheep IgM ELISA Kit	31.25 ppb
	DEIA-BY048	Turkey IgG ELISA Kit	1.563 ppb
	DEIA-BY049	Turkey IgM ELISA Kit	3.13 ppb

Antibiotics

In the cell culture process, the use of appropriate concentrations of antibiotics can prevent contamination and the morphological or physiological changes caused by contamination. In addition, certain antibiotics are also used to screen transfected/genetically modified cells. Therefore, antibiotics are common cell culture-derived impurities in the biomanufacturing process. The following products can be used to detect antibiotic impurities in cell culture medium, fermentation fluid, vaccine supernatant, DNA TE buffer and other samples with high sensitivity, and are suitable for high-throughput quantitative screening of samples.

Ralated Product

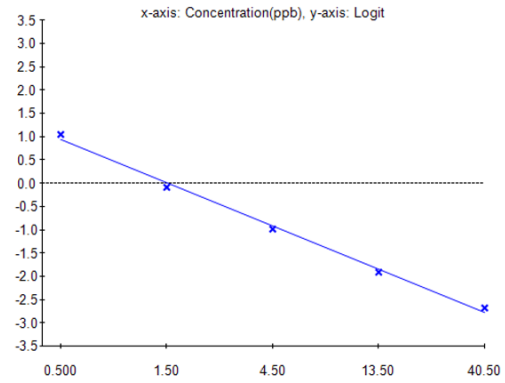
Analytes	Cat. No	Product Name	Sensitivity
Carbenicillin	DEIA-BY013	Carbenicillin ELISA Kit	Under development
Chloramphenicol	DEIA018	Chloramphenicol, CAP ELISA Kit	0.025 ppb
Colistin	DEIA042	Colistin ELISA Kit	1.5 ppb
Geneticin	DEIA-BY014	Genetici ELISA Kit	Under development
Gentamicin	★DEIA047	Gentamicin ELISA Kit	0.1 ppb
Kanamycin	★DEIA048	Kanamycin ELISA Kit	0.5 ppb
	★DEIA-WZ048V	High Sensitivity Kanamycin ELISA Test Kit	0.2 ppb
	DEIA048V	Kanamycin ELISA Kit	0.05 ppb
Meropenem	DEIA-BY015	Meropenem ELISA Kit	Under development
Methotrexate	★DEIA-XYZ209	Methotrexate ELISA Kit	0.16 ppb
Penicillin	DEIABL-QB24	Penicillin ELISA Kit	0.1 ppb
	DEIABL-QB25	Benzyl penicillin ELISA Kit	0.1 ppb
Puromycin	DEIA-BY016	Puromycin ELISA Kit	Under development
Streptomycin	★DEIA020	Streptomycin ELISA Kit	0.1 ppb
Tetracycline	DEIA046	Tetracyclines ELISA Kit	0.2 ppb

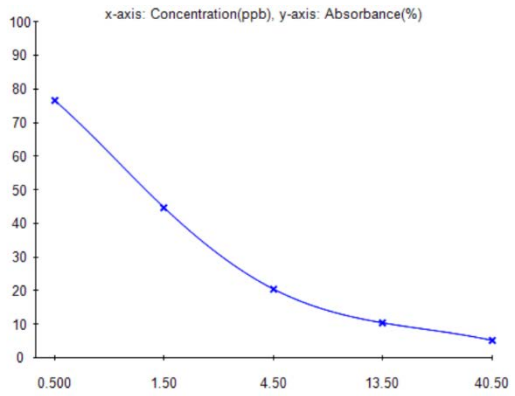
Thiaphenicol	DEIA-BY017	Thiaphenicol ELISA Kit	Under development
Tobramycin	DEIA-BY018	Tobramycin ELISA Kit	Under development
Vancomycin	DEIANJ11	Human Vancomycin ELISA Kit	0.5 ppb

(Catalog number marked with ★ are hot products)

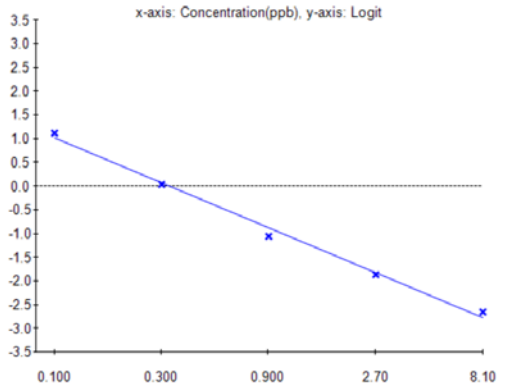
After many tests, we obtained the parameters of the kit, as shown in the following Data Example:

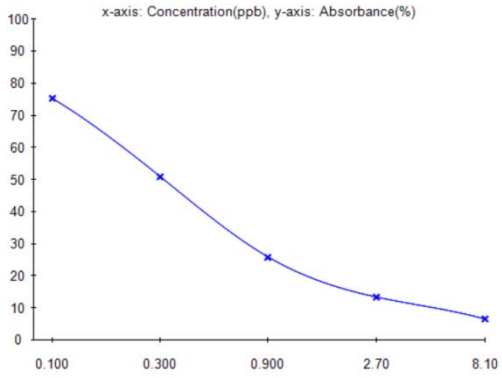
Cat. No	Product Name	Detection Range	IC50	CV	Recovery	Linearity
DEIA048	Kanamycin ELISA Kit	0.5-40.5 ppb	1.2 ppb	⩽10%	81.2% to 102%	0.99





Cat. No	Product Name	Detection Range	IC50	CV	Recovery	Linearity
DEIA047	Gentamicin ELISA Kit	0.1-8.1 ng/mL	0.442 ppb	⩽10%	86% to 119.7%	0.99





PEG

The modification of biopharmaceutical molecules by covalent conjugation of polyethylene glycol (PEG) molecules is known to improve the pharmacological and pharmaceutical properties of proteins and other large molecules. Frequently, one end of the polymer is capped with a methoxy group (mPEG) to prevent unwanted cross-linking during conjugation. Residual impurities associated with such processing additives must also be removed from the API or the final drug product.

Ralated Product

Category	Cat. No	Product Name	Sensitivity
For detection of PEGylated proteins	DEIA6158	High Sensitivity Polyethylene Glycol (PEG) ELISA Kit	0.064 ppb
	DEIABL235	mPEG ELISA Kit	0.3125 ppb
For detection of PEG antibody	DEIA6159	Mouse anti-PEG IgG ELISA Kit	3.13 U/mL
	DEIA6160	Mouse anti-PEG IgM ELISA Kit	6.25 U/mL
	DEIASL085	Rat anti-PEG IgG ELISA Kit	6.25 U/mL
	DEIASL086	Rat anti-PEG IgM ELISA Kit	3.13 U/mL
	DEIASL087	Monkey anti-PEG IgG ELISA Kit	1.56 U/mL
	DEIASL088	Monkey anti-PEG IgM ELISA Kit	6.25 U/mL
	DEIA-BY029	Rabbit Anti-PEG IgG ELISA Kit	0.78 U/mL
	DEIA-BY030	Rabbit Anti-PEG IgM ELISA Kit	1.56 U/mL
	DEIASL243	Human Anti-PEG IgG ELISA Kit	0.78 U/mL
	DEIASL244	Human Anti-PEG IgM ELISA Kit	3.13 U/mL

Downstream-derived impurities

Any API purification process can also introduce additional process-related impurities into the biopharmaceutical product. Potential downstream impurities such as enzymes, processing reagents (e.g., cyanogen bromide, guanidine, oxidizing and reducing agents), leached metallic elements (e.g., leached from a metal-complex chromatographic resin), leached ligands (e.g., leached from a Protein A affinity column resin), and volatile organic solvents (from chromatography solvents), must all be evaluated.

Ralated Product

Protein A

Cat. No	Product Name	Sensitivity
DEIA6474	Protein A ELISA Kit	0.05 ppb
DEIA-BY019	Alkali-Resistant Protein A ELISA Kit	6.6 ppt
DEIA-BY020	Recombinant Protein A ELISA Kit	7 ppt

Bioprocessing enzymes

Analyte	Cat. No	Product Name	Sensitivity
Benzonase	DEIA-JY2103	Benzonase ELISA Kit	0.05 ppb
Endonuclease	DEIA-BY024	Endonuclease ELISA Kit	0.06 ppb
Vaccinia Capping Enzyme	DEIA-BY031	Vaccinia Capping Enzyme ELISA Kit	0.05 ppb
2'-O- Methyltransferase	DEIA-BY032	2'-O- Methyltransferase ELISA Kit	0.05 ppb
DNASE1	DEIANS026	DNase I ELISA Kit	0.05 ppb
T7 RNA Polymerase	DEIANS032	T7 RNA Polymerase ELISA Kit	0.1 ppb
RNase Inhibitor	DEIANS033	RNase Inhibitor ELISA Kit	5 ppb

Immunotoxicity

Analyte	Cat. No	Product Name	Sensitivity
KLH	DEIA3834	KLH IgG (Mouse) ELISA Kit	6.25 U/mL
	DEIASL066	KLH IgM (Mouse) ELISA Kit	6.25 U/mL
	DEIASL067	KLH IgG (Rat) ELISA Kit	15.63 ppb
	DEIASL068	KLH IgM (Rat) ELISA Kit	7.81 ppb
	DEIASL069	KLH IgG1 (Monkey) ELISA Kit	0.94 ppb
	DEIASL070	KLH IgM (Monkey) ELISA Kit	12.5 ppb
	DEIA3833	KLH IgG (Guinea Pig) ELISA Kit	6.25 U/mL
	DEIA-BY027	Human Anti-KLH IgG ELISA Kit	0.94 ppb
	DEIA-BY028	Human Anti-KLH IgM ELISA Kit	31.25 ppb
Tetanus Toxoid	DEIASL075	Rat Tetanus Toxoid IgG ELISA Kit	0.39 U/mL
	DEIASL076	Rat Tetanus Toxoid IgM ELISA Kit	3.13 U/mL
	DEIASL077	Monkey Tetanus Toxoid IgG1 ELISA Kit	0.078 ppm
	DEIASL078	Monkey Tetanus Toxoid IgM ELISA Kit	3.91 ppb
	DEIASL264	Mouse Anti-Tetanus Toxoid IgG ELISA Kit	Under development

	DEIASL273	Rabbit Anti-Tetanus Toxoid IgG ELISA Kit	Under development
	DEIA378	Human Tetanus Toxoid IgG ELISA Kit	0.001 IU/mL
	DEIA2568	Human Anti-Tetanus Toxoid ELISA Kit	0.001 IU/mL
Dinitrophenol	DEIASL081	Dinitropheno (Mouse) IgG ELISA Kit	3.125 U/mL
	DEIASL082	Dinitropheno (Mouse) IgM ELISA Kit	3.125 U/mL
	DEIASL083	Dinitropheno (Rat) IgG ELISA Kit	3.125 U/mL
	DEIASL084	Dinitropheno (Rat) IgM ELISA Kit	3.125 U/mL
Trinitrophenol	DEIASL079	Trinitrophenol (Mouse) IgG ELISA Kit	3.125 U/mL
	DEIASL080	Trinitrophenol (Mouse) IgM ELISA Kit	3.125 U/mL

Features

- High sensitivity—IC50 of the kit could reach ppb level.
- High specificity—Cross-reactivity with other antibiotics is less than 0.1%.
- High reproducibility—Coefficients of variation between and within batches were <10%.
- The standard curves with great linearity were obtained (Note: The standard curve was generated in our lab for demonstration purposes only. Users should obtain the standard curve according to their own experiments.).
- High production quality

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