



**SARSUAG ENTERPRISES**



**Consultancy  
Services**

**Solutions for Life Science Research**

**March 2018**

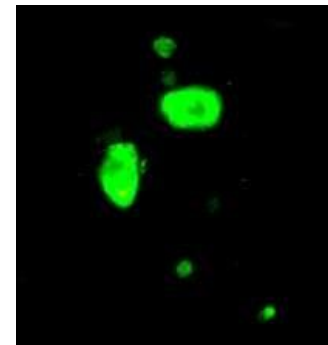
# Sarsuag Enterprises



Science-focused biotechnology company established in 2016.



Offering value-added contract research services for Life Sciences R&D.



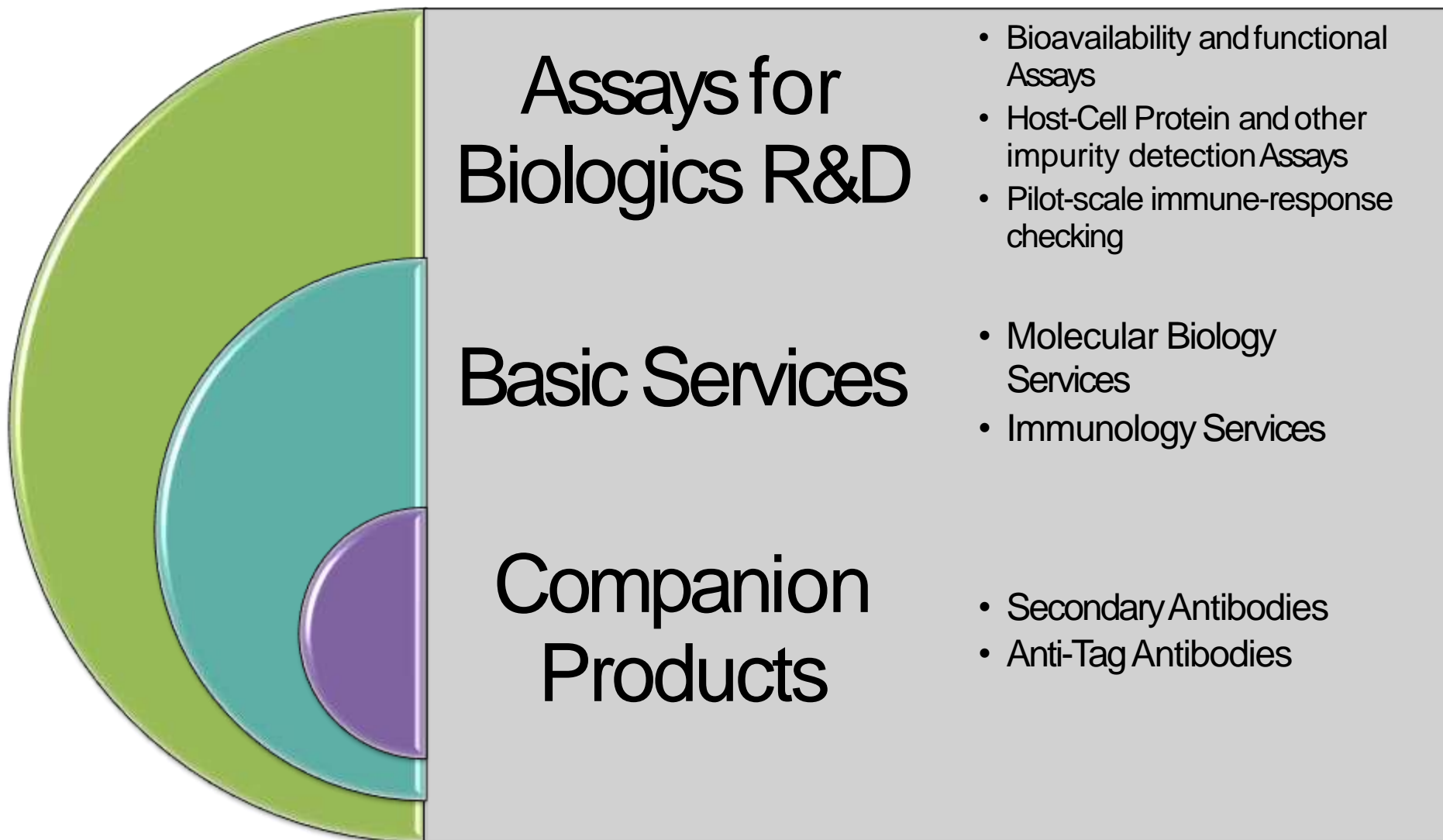
Founder of company have 15 years of experience in Biotech and CRO Industries



Cutting-edge science providing fast solutions with best-in-class quality.



# Contract Research Offerings



# Immunology Services: Antibody Development



*Custom Antibodies against Protein, Peptides, and mixture of proteins*

**Polyclonal Antibody**  
Antibodies in Rabbit  
and Goat

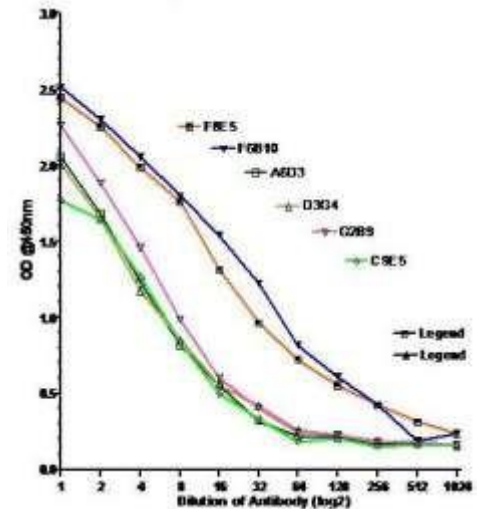
**Monoclonal Antibody**  
Hybridoma  
Development in Mouse

## Antibody Purification

Affinity Purification –  
Protein A/G

Antigen-affinity for Mono-specific pAbs

Double-affinity purification (for modified peptides)



*Example: Dilution curve of antibody clones to demonstrate relative affinity*

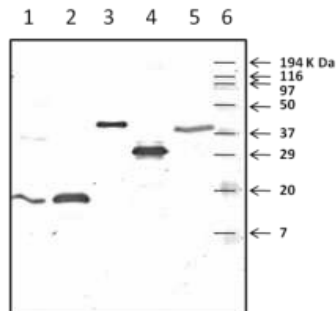
## Capability Highlights

- ✓ Analysis-driven development process – all antigens do not have same immune response
- ✓ Faster development for immunogenic antigens
- ✓ Multitude of purification columns to optimize yield and affinity

# Immunology Services: Antibody Characterization

## Immuno-assay Development

- Western Blot
- Immuno-precipitation
- Immuno-cytochemistry
- Immuno-histochemistry (on human and animal tissues)
- Immuno-Fluorescence
- Sandwich ELISA Pair Determination and Prototyping



## Antibody Conjugation

With HRP, FITC, Biotin, ALP or any other conjugate / fluorophore

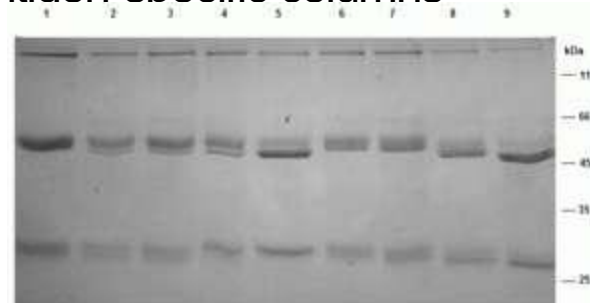
## Example: IHC of Thyroglobulin and EMA antibody on human tissues



*We have relationship with clinics in Bangalore and Chennai for sourcing human samples*

## Immuno-Purification

Purification from serum on Protein-A or Antigen-specific columns



# Assays for Biologics R&D

## Bioavailability and Functional Assays

- Custom-designed antibodies and immunoassays to detect the product in serum at a specified limit
- Other functional immunoassays for product characterization

## Process-Related Impurity Detection Assays

- Custom-designed antibodies and immunoassays for **Host-Cell Protein** detection
- Hybridization-based assays for **Host-Cell DNA** detection
- Custom immunoassays for other process-derived contaminants such as growth factor, media etc

## Immunogenicity assay for Testing Clinical Samples

- Development of reagents for Assay
- Development and Validation of Immunogenicity Assay
- Analysis of Clinical samples in GLP Environment

## Process-Related Impurity Detection

- ❑ For any biopharmaceutical product, the purification process of the target active protein may co-purify other contaminants from the cell
  
- ❑ Such contaminants may have immunogenic properties affecting the patient safety and product consistency
  
- ❑ Need an assay system to detect contaminants such as:
  - Host Cell Protein
  - Host Cell DNA
  - Growth factors and other media used in cell culture
  
- ❑ These impurities are characteristics of the process and not of the product itself

# Process-related Impurities: Regulatory View

## Excerpt from ICH Q6B (Test Procedure and Acceptance Criteria for Biotechnological Products)

*“Cell substrate-derived impurities include, but are not limited to, proteins derived from the host organism, nucleic acid (host cell genomic, vector, or total DNA).*

*“For host cell proteins, a sensitive assay e.g., immunoassay, capable of detecting a wide range of protein impurities is generally utilized. In the case of an immunoassay, a polyclonal antibody used in the test is generated by immunization with a preparation of a production cell minus the product-coding gene, fusion partners, or other appropriate cell lines.*

*“The level of DNA from the host cells can be detected by direct analysis on the product (such as hybridization techniques).*

*“Clearance studies, which could include spiking experiments at the laboratory scale, to demonstrate the removal of cell substrate-derived impurities such as nucleic acids and host cell proteins may sometimes be used to eliminate the need for establishing acceptance criteria for these impurities.”*



# Process-related Impurity Detection : Our Offerings

## Host-Cell Protein

- Polyclonal antibody development (validation using 1D and 2D SDS-PAGE)
- Assay in Western Blot or ELISA format
- Validation in Spiked Recovery Assay

## Host-Cell DNA

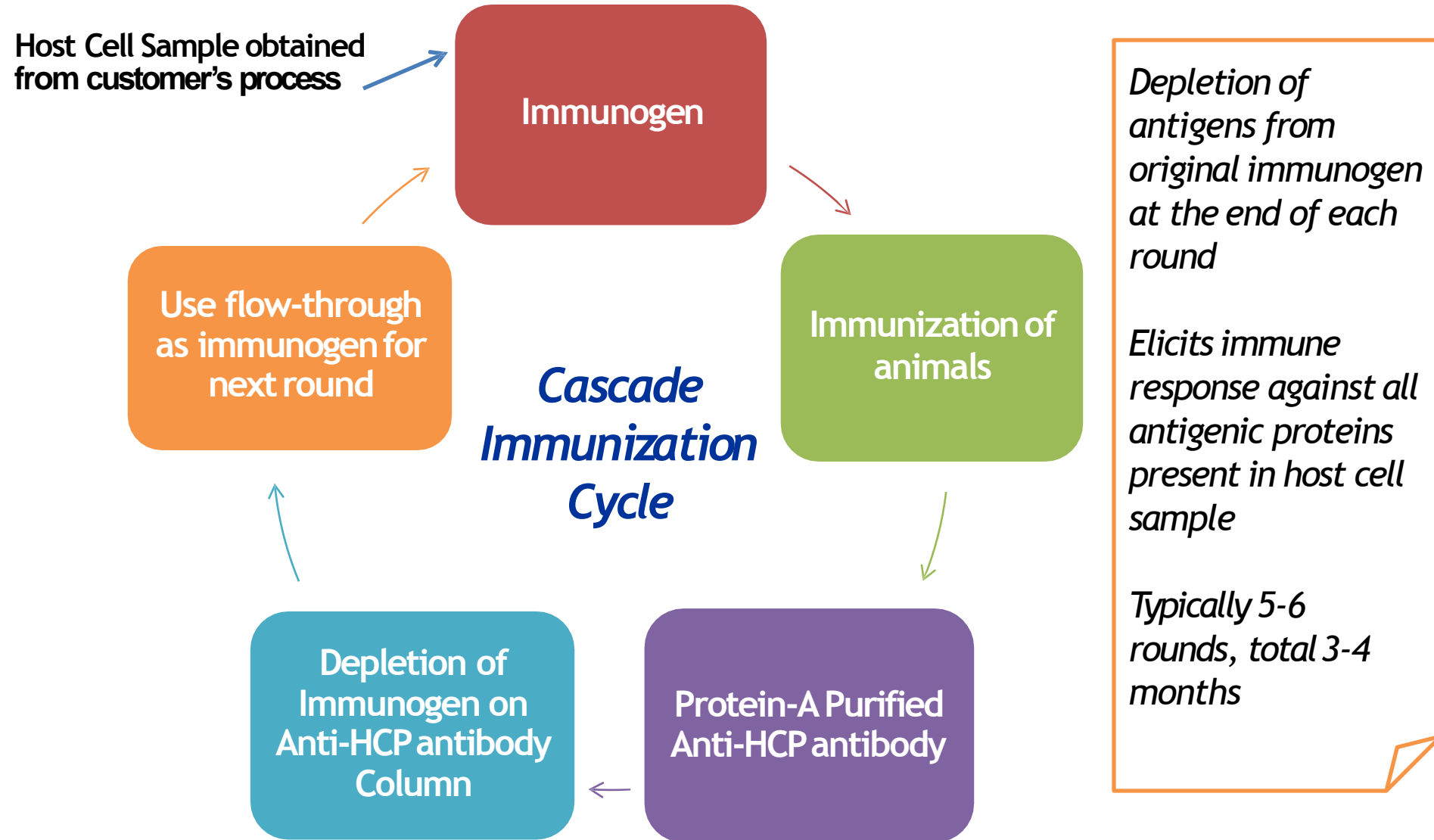
- PCR amplification using Random Primers
- Detection using hybridization (with DIG-labeled probe)
- Validation in Spiked Recovery Assay

## Culture Contaminants

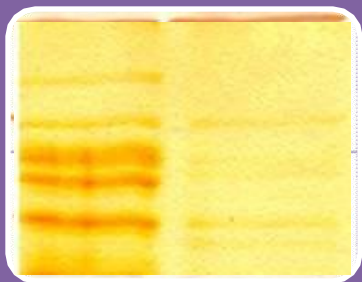
- Custom immunoassay against specific contaminant
- E.g. we already have a Sandwich ELISA assay for Insulin
- Validation in Spiked Recovery Assay

***Assay strategies that have been generally acceptable to FDA biotechnology product reviewers***

# HCP Antibody Development

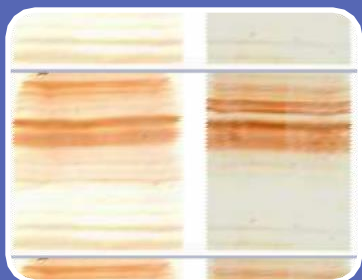


# Method Validation



## Antigen Depletion

- Run depleted immunogen at the end of each round on SDS-PAGE
- Silver stain and compare with original immunogen to validate antigen depletion



## Antibody Response

- Run original immunogen in multiple lanes and do western blotting at the end of each round with collected antisera
- Appearance of new bands in Western Blot validates antibody response for less immunogenic antigens in host cell sample

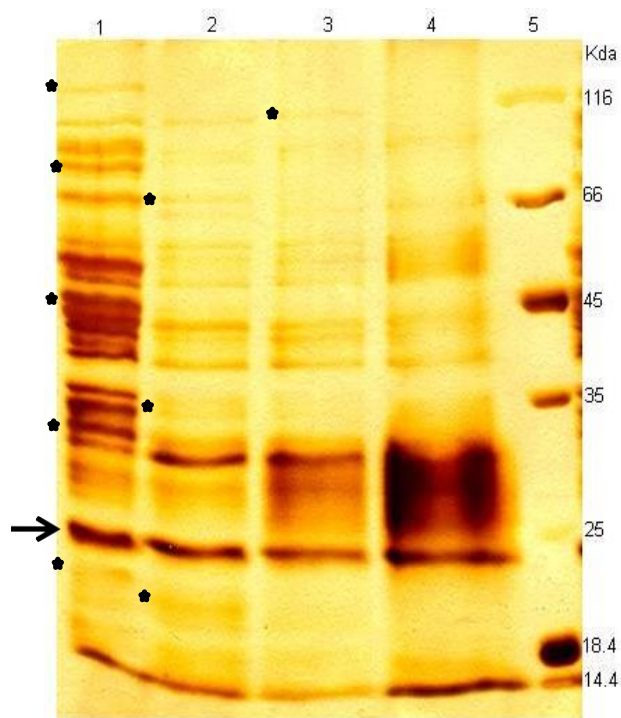


## Completeness

- Run original immunogen in 2D gel and do western blotting at the end of each round with collected antisera
- Appearance of new spots in Western Blot validates antibody response for less immunogenic antigens in host cell sample

# Antigen depletion of Immunogens

## Silver staining of 15% SDS PAGE

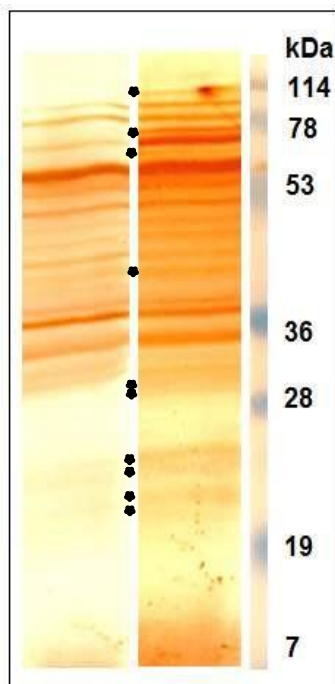


Lane 1: Total cell lysate- 50  $\mu$ g  
 Lane 2: 1<sup>st</sup> Depletion – 50  $\mu$ g,  
 Lane 3: 2<sup>nd</sup> Depletion – 50  $\mu$ g,  
 Lane 4: 3<sup>rd</sup> Depletion- 50  $\mu$ g .

1. Passage over anti-HCP column depletes highly immunogenic proteins ( ★ )
2. Less immunogenic Proteins ( → ) are not depleted and is enriched by sequential passage over the anti-HCP column
3. During sequential depletions, the number of differential loss of protein bands decreases.
4. Depletion using the third bleed anti-HCP column did not show significant loss of protein bands as compared to the second depletion, indicate the depletion of all immunogenic proteins is complete.

# Cascade Immunizations

## 1-D Western Analysis



Lane 1: 100  $\mu$ g total HCP detected with 1<sup>st</sup> bleed anti-HCP antibody

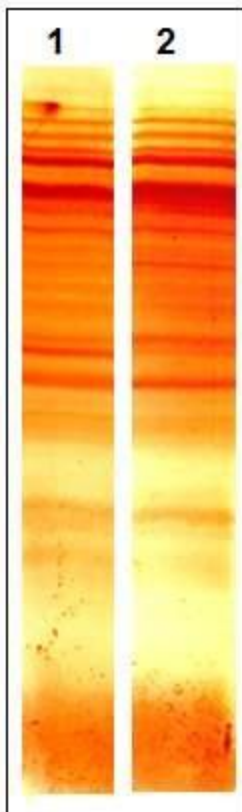
Lane 2: 100  $\mu$ g total HCP detected with 1-3<sup>rd</sup> pooled bleed anti-HCP antibodies

For detection HRP conjugated anti-Rabbit secondary antibodies were used and blot developed using DAB

**Three sequential depletions resulted in at least two-fold increase in the number of proteins against antibodies were raised**

# Antibody response is sustained through Cascade Immunization schedule

1 D western analysis



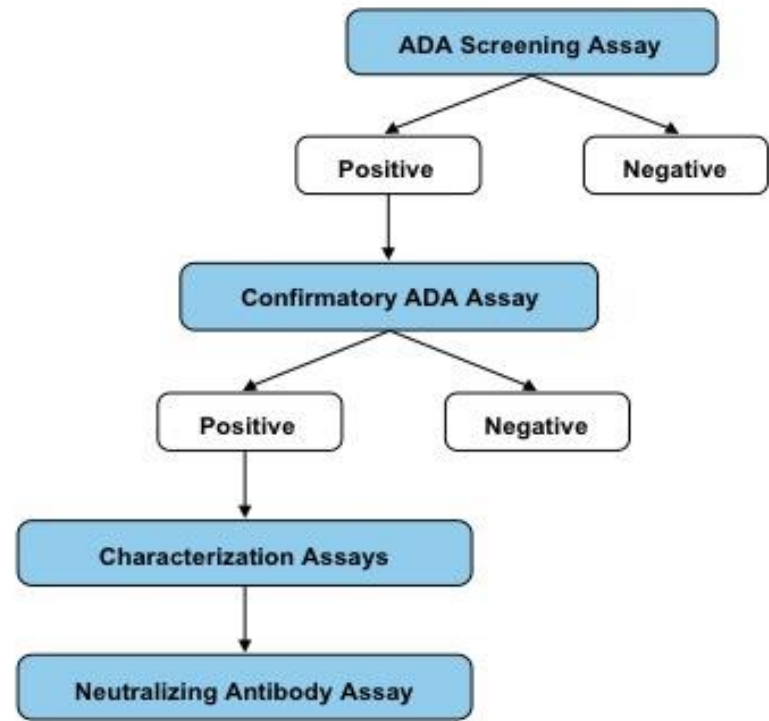
Lane 1: 100ug total HCP detected with pooled 1-3<sup>rd</sup> bleed anti-HCP antibody  
 Lane 2: 100ug total HCP detected with 3<sup>rd</sup> bleed anti- HCP antibodies  
 For detection HRP conjugated anti-Rabbit secondary antibodies were used and blot developed using DAB

**The profile of protein bands detected by the 3<sup>rd</sup> bleed antibodies is similar to that observed by the pooled 1<sup>st</sup> - 3<sup>rd</sup> bleed antibodies**

# Immunogenicity Assay for clinical Development of Biosimilars

## Immunogenicity

- Development and Validation of Method according to EMEA and FDA guideline.
- Analysis of Samples in GLP controlled environment.
- Development and validation of Method for Neutralizing Antibodies.



## Platform

**Sandwich /Bridge  
ELISA**

**CELL BASED ASSAY**

## PHARMACOKINETICS ASSAY FOR BIOSIMILARS

### Pharmacokinetic Assays

**We have industry leading experience in development and Validation of PK/TK assay for large molecules and peptides.**

- Develop and validate assays that have analytical ranges which appropriate for the study samples.
- Develop assay that are both specific and sensitive
- Provide support for critical reagent generation and purification including the generation of antibodies to be used for capture and detection reagents.
- Assess method feasibility in multiple technology platforms.



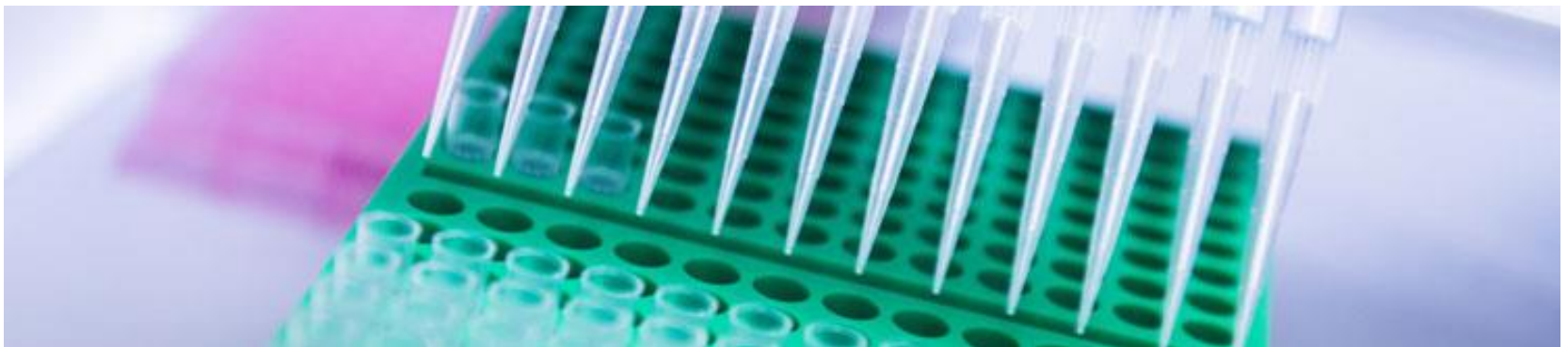
Sarsuag's Innovate assay methods to address even most challenging requirements.

### Cell-based assay

- **ADCC & CDC**
- **Reporter ADCC**
- **Potency**

### Binding Assays

- **Target Affinity (SPR & ITC)**
- **Target Binding ELISA**
- **Target Cellular Binding**



# Bioanalytical Method for Biosimilar/Biologics

Parameter	Assay
Identity	SDS-PAGE (non-reduced; EP) Western-Blot Peptide map N-terminal sequencing
Purity / Integrity(Degradation or aggregation products)	SDS-PAGE (reduced)Western-Blot and HP-SEC
Isoforms(EP)	IEF
Content	ELISA OD 280/320 nm
Glycosylationpattern	HPAEC-PAD (native oligosaccharides; neutralstructures)
Endotoxin	LAL
Potency	Bio assay

Parameter	Assay
Monomer content	HPLC-SEC (EP) or Gel filtration reduced
Primary structure	ESI-MS
Tertiary structure	CD spectrum
Site specific glycosylation	ESI/TOF-MS; MALDI/TOF-MS
Oxidation analysis	LC-ESI-MS
MethionineOxidation	RP-HPLC or Peptide Map
N-terminal sequence	ESI-MS, ESI-MS-MS or Edman N terminal seq
C-terminal heterogeneity	Carboxypeptidase
Tryptic Peptide Mapping(EP)	HPLC

# TOXICOLOGY STUDY THROUGH OUR PARTNER (Liveon Biolabs )

- General Toxicology**
- ❖ Acute Toxicity
  - ❖ Sub Acute Toxicity
  - ❖ Sub Chronic Toxicity
  - ❖ Chronic Toxicity

- Special Toxicity**
- ❖ Toxicokinetics
  - ❖ Antibacterial activities
  - ❖ Carcinogenicity
  - ❖ Immunotoxicology

- Local Irritation**
- ❖ Dermal Irritation
  - ❖ Local Lymph Node Assay
  - ❖ Photosensitivity
  - ❖ Skin Irritation
  - ❖ Skin Sensitization

- Genetic Toxicity**
- ❖ AMES
  - ❖ Micronucleus Assay (In vivo)
  - ❖ Chromosomal Aberration (in-vivo and in-vitro)
  - ❖ Cytotoxicity

- Reproductive Toxicity**
- ❖ Prenatal Developmental
  - ❖ One-Generation Reproduction
  - ❖ Two-Generation Reproduction
  - ❖ Reproduction-Developmental Toxicity

- Biocompatibility Studies  
( Medical Devices)**
- ❖ Cytotoxicity
  - ❖ Skin Sensitization
  - ❖ Acute Systemic Toxicity
  - ❖ Subchronic Toxicity
  - ❖ Chronic Toxicity
  - ❖ Irritation / Intracutaneous Reactivity
  - ❖ Genotoxicity
  - ❖ Implantation
  - ❖ Hemocompatibility
  - ❖ Carcinogenicity
  - ❖ Reproductive Toxicity

**Note : We have Partner in Canada and USA to conduct toxicology study in Monkey**

# Antibodies for Biosimilars Development



Rabbit anti-Trypsin	: SS-I-110-A
Rabbit Anti-Trypsin-HRP	: SS-I-110-B
Rabbit Anti Human TNF alpha Antibody	: SS-I-111
Rabbit Anti Etanercept Antibody	: SS-I-112
Anti Rituximab Monoclonal Antibody	: SS-I-113
Rabbit Anti Adalimumab Antibody	: SS-I-114
Anti TNF alpha Monoclonal Antibody	: SS-I-115
Rabbit Anti-Bevacizumab Antibody	: SS-I-116
Anti-GCSF Monoclonal Antibody	: SS-I-117
Anti-PEG Monoclonal Antibody	: SS-I-118
Anti-h Alpha Subunit Monoclonal Antibody	: SS-I-119
Anti-hCG Monoclonal Antibody	: SS-I-120
Anti-FSH Monoclonal Antibody	: SS-I-121
Anti-LH Monoclonal Antibody	: SS-I-122
Rabbit Anti-Ranibizumab Antibody	: SS-I-123
Rabbit Anti-Rituximab Antibody	: SS-I-124
Rabbit Anti-Trastuzumab Antibody	: SS-I-125
Rabbit Anti-Liraglutide Antibody	: SS-I-126

## **IMMUNOGENICITY ELISA KITS**

Etanercept (Enbrel) Immunogenicity KIT (SS-E-101)  
Rituximab (Mabthera) Immunogenicity KIT (SS-E-102)  
Bevacizumab (Avastin) Immunogenicity KIT (SS-E-103)  
Ranibizumab (Lucentis) Immunogenicity KIT (SS-E-104)  
Trastuzumab (Herceptin) Immunogenicity KIT (SS-E-105)

## **PK ELISA KITS**

Infliximab (Remicade ) PK ELISA KIT (SS-E-106)  
Infliximab ( Remicade ) PK ELISA KIT (SS-E-107)  
Adalimumab (Humira ) PK ELISA KIT (SS-E-108)  
Adalimumab (Humira ) PK ELISA KIT (SS-E-109)  
Etanercept ( Enbrel ) PK ELISA KIT (SS-E-110)  
Rituximab (Mabthera ) PK ELISA KIT (SS-E-111)  
Ranibizumab (Lucentis ) PK ELISA KIT (SS-E-112)  
Trastuzumab ( Herceptin ) PK ELISA KIT (SS-E-113)

## **CYTOKINE & HCP KITS**

Human TNF alpha ELISA KIT (SS-E-114)

Human TNF alpha ELISA KIT (SS-E-115), Without Pre-coated Plate

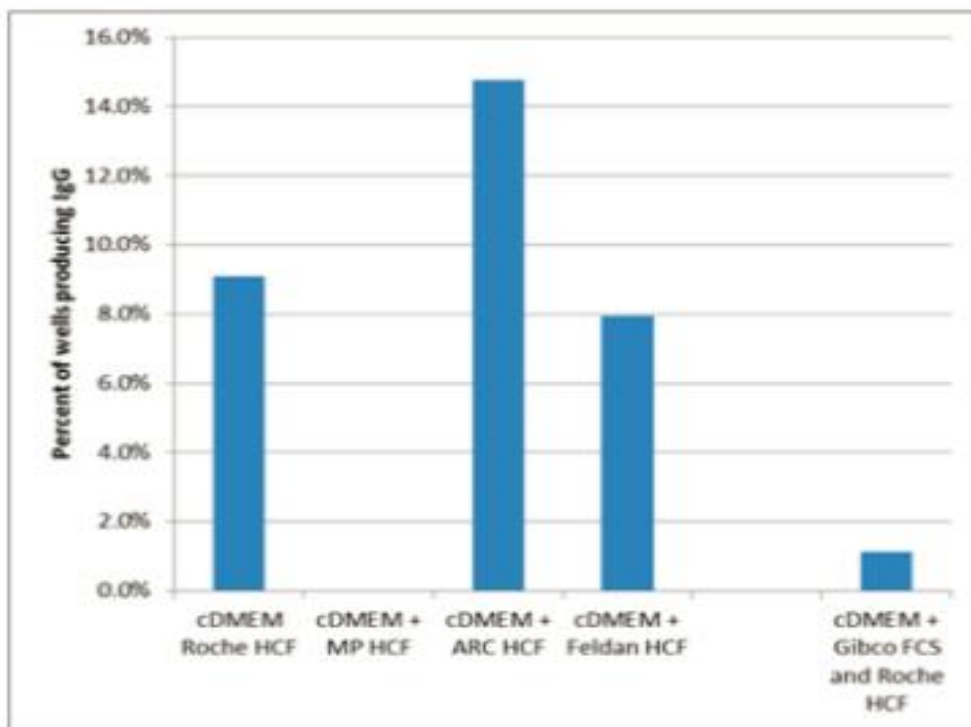
DeQuanto™ E. Coli HCP ELISA KIT (SS-E-116)

DeQuanto™ CHO HCP ELISA KIT (SS-E-116)

## PRODUCTS FROM OUR PARTNER (Antibody Research corporation, USA)

### HyMax™ SF Hybridoma Fusion & Cloning Supplement Serum Free 20x

Third party independent comparisons of HyMax to other products:





# CELL LINES FOR RESEARCH AND DEVELOPMENT

(Antibody Research corporation, USA)

[Ag8 Cells \(Mouse Myeloma\)](#)

[BHK21 cell line \(hamster kidney cells\)](#)

[BW1100 \(T cell Thymoma\)](#)

[CaCO2 cell line \(human colorectal adenocarcinoma\)](#)

[CHO cell line \(hamster ovary cells\)](#)

[COS1 cell line \(monkey SV40 transformed kidney cells\)](#)

[Daudi cell line \(human lymphoma, Burkitt\)](#)

[EL4 cell line \(mouse lymphoma\)](#)

[HaCaT cell line \(human keratinocytes\)](#)

[HEK293 cell line \(human embryonic kidney\)](#)

[HeLa cell line \(human cervical carcinoma\)](#)

[Hep2 cell line \(human larynx squamous cell carcinoma\)](#)

[HepG2 cell line \(human hepatocellular carcinoma\)](#)

[HT-2 \(mouse T cells\)](#)

[Huv-Ec-C cell line \(human vascular endothelium\)](#)

[J774.1 cell line \(mouse monocyte/macrophage\)](#)

[Jurkat cell line \(human T cell leukemia\)](#)

[K562 cell line \(human chronic myelogenous leukemia \)](#)

[KB cell line \(human\)](#)

[L929 cell line \(mouse subcutaneous connective tissue\)](#)

# CUSTOMIZED INSTRUMENTS FROM SARSUAG

**Incubator**  
Cat.No : SS-I-105



**UVS Ultra Portable Lamp**  
Cat.No : SS-I-106



**UVHL Portable  
Lamp**



**Vertical Gel  
Electrophoresis Unit (SDS  
- PAGE)**



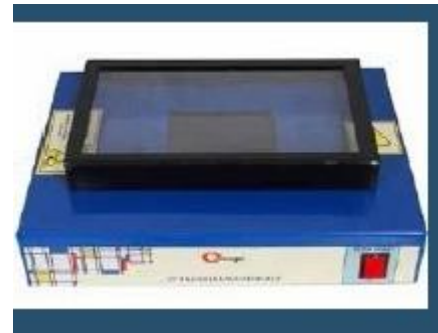
# SARSUAG INSTRUMENTS



**Electrophoresis system**  
Cat. No : SS-I-101



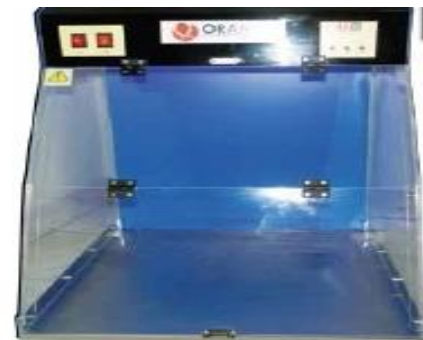
**UV Transilluminator**  
Cat. No : SS-I-102



**Gel Rocker**  
Cat. No : SS-I-103



**PCR Work Hood**  
Cat. No : SS-I-104



## SARSUAG INSTRUMENTS

**Rotary evaporator**  
Cat.No : SS-I-109



**Magnetic stirrer**  
Cat.No : SS-I-110



**Overhead stirrer**  
Cat.No : SS-I-111



**Micro centrifuge**  
Cat.No : SS-I-112 & Cat.No : SS-I-113



## SARSUAG INSTRUMENTS

LCD digital shaker  
Cat.No : SS-I-114



Micro plate Mixer Cat.No : SS-I-



LCD digital rotator  
Cat.No : SS-I-116



LCD digital tube roller  
Cat.No : SS-I-117



**Programmable Power  
Supplies Cat.No : SS-I-118**



**Autoclaves Vertical : SS-I-119**



**Autoclaves Horizontal : SS-I-**



**Circulating Water Baths :  
Cat.No : SS-I-121**



**Humidity / Stability  
Chambers Cat.No : SS-I-122**



**Programmable Oven : SS-I-**



**Cryo Box (Any Size) : SS-I-123**



**Bio-Safety Cabinets :  
Cat.No : SS-I-125**



**Stackable Refrigerated  
Incubator Shaker Cat.No : SS-**



**UV/VIS Spectrophotometer  
Cat.No: SS-I-127**



**Hygene Bioreactor /  
Fermentor**



**CO2 Incubator : Cat.No : SS-I-**





Refrigerated Centrifuge  
Cat.No : SS-I-130



Freeze Dryer - Lyophilizer  
Cat.No: SS-I-131

Compact & Multitask Lyophilizer



Freeze Dryer

Sonicator : SS-I-132

Novel Appearance  
& Reliable Performance



Ice Flake Machine : Cat.No : SS-I-



**Double Decker Refrigerated Shaker Cat.No : SS-I-134**



**Refrigerated Shaker – Advance Model**



**Incubator Shaker : SS-I-136**



**INCUBATOR SHAKER**

**Ultrasonic Bath : Cat.No : SS-I-137**



## PHD RESEARCH WORK

- We undertake PhD research work in field of Molecular Biology, Immunology , Biophysics and Molecular Biology.
- We also train researcher and scientist on different techniques like stem cell therapy and novel drug delivery system.
- We encourage researcher to published national and International Journal.

## Why Sarsuag Enterprises ??

- One stop shop for all biology solutions and Lab Instruments
- Flexible service model to meet your requirements
- Offsite development – without burdening customers infrastructure

### √Our Values

- Quality
- Speed
- Integrity

√ We provide best science for your business

# We are working for....

## **Pharma & Biotech**

Intas Biopharma, Ahmedabad

Zydus Cadila, Ahmedabad

Sun Pharma, Vadodara

Unichem Laboratories, Goa

Biocon, Bangalore

## **Global Solution Providers**

Antibody Research corporation, USA

## **Our Partners**

Antibody Research corporation, USA

Liveon Biolabs Pvt. Ltd. Bangalore

# Thank You

## **SARSUAG ENTERPRISES**

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