2026 Sakura Science Programm~Osaka of University

BIOPHARMACEUTICAL MANUFACTURING



GROUP 2

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CONTENT

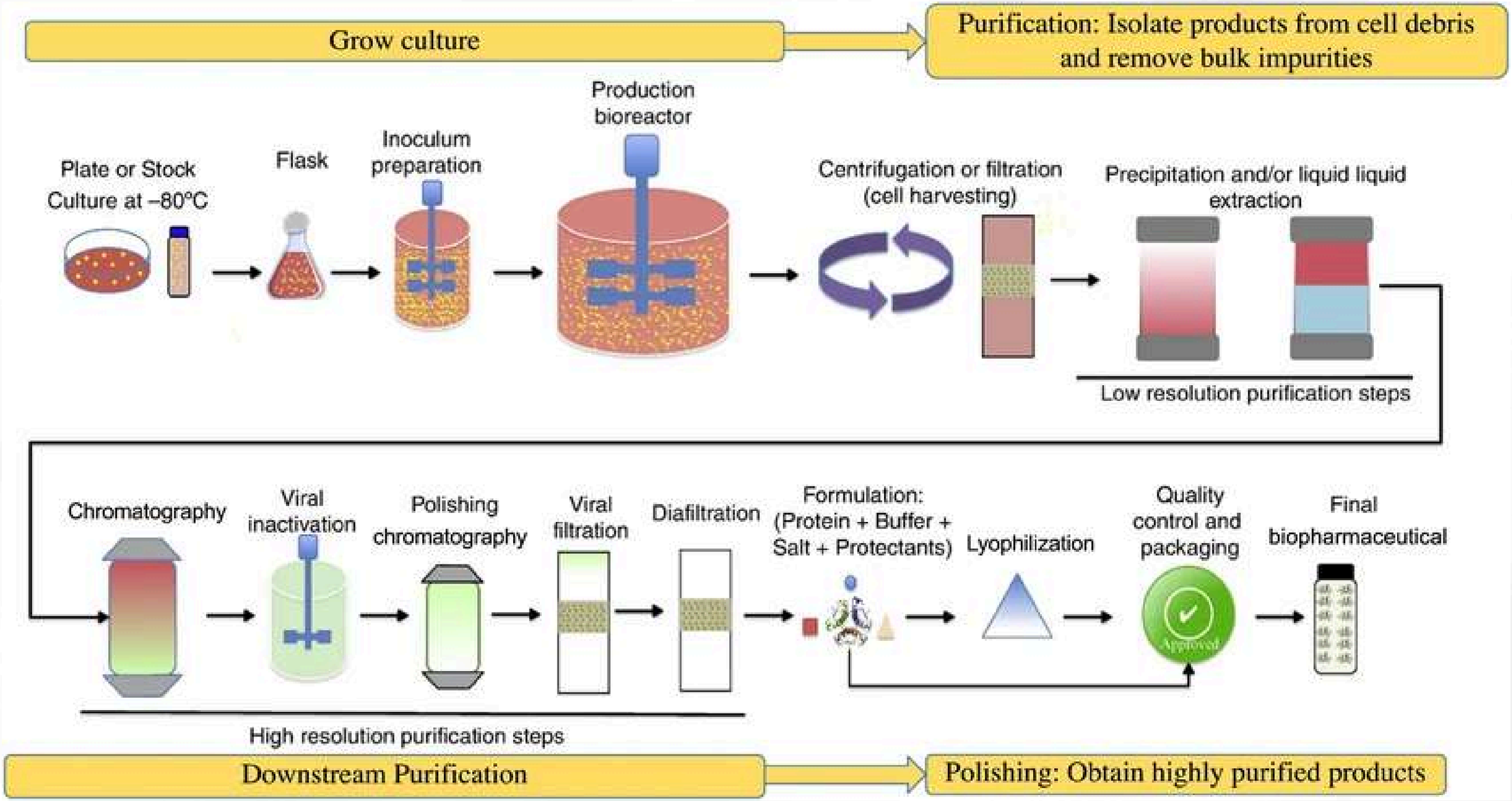
01 Manufacturing processes

02 Manufacturing equipments

03 Best selling Biopharmaceuticals in current market

04 IgGs Separation by SDS-PAGE

OVERALL MANUFACTURING PROCESS



MANUFACTURING STAGES

STAGE 01

Upstream

Grow cells and synthesize the therapeutic protein



STAGE 02

Harvest

Remove cells and debris from the product-containing liquid



STAGE 03

Downstream

Remove impurities; concentrate and exchange buffer



STAGE 04

Fill-Finish

Prepare and package the finished medicine



UPSTREAM PROCESSING

STAGE 01



Bioreactor equipment

WHAT HAPPENS IN UPSTREAM?

01 Cell bank

A characterized CHO cell line is recovered.

02 Seed train

Cells are expanded to sufficient volume.

03 Production bioreactor

Cells secrete IgG into culture medium.

04 Process controls

pH, temperature, dissolved O_2 and nutrients are monitored.

OUTPUT → IgG-containing culture broth

HARVEST & CLARIFICATION

STAGE 02

GOAL **Remove CHO cells and debris while keeping soluble IgG in the harvest fluid.**

01 Centrifugation

Separates most intact cells and larger solids from the culture liquid.

02 Depth filtration

Reduces residual particles and turbidity before capture purification.



KEEP **Clarified supernatant containing soluble IgG**

Harvest clarifies the broth; it is not the final purification step.

DOWNSTREAM: PROTEIN A CAPTURE

STAGE 03

Why Protein A? **Protein A binds the Fc region of many IgG antibodies.**

01 LOAD

Clarified harvest passes through the Protein A resin.

02 WASH

Many nonbinding process impurities flow through.

03 ELUTE

Changing solution conditions releases enriched IgG.

04 VIRAL INACTIVATION

A validated low-pH hold can inactivate many enveloped viruses.

CAPTURE → Enriched IgG; additional purification remains necessary.

DOWNSTREAM: POLISHING & UF/DF

STAGE 03

01 Polishing chromatography

Further reduce host-cell proteins, residual DNA, aggregates and leached Protein A.

02 Virus-removal filtration

Use a validated filter step to reduce viral particles.

03 Ultrafiltration / diafiltration

Concentrate IgG and replace the surrounding buffer.



UF/DF processing area

OUTPUT → Purified and concentrated IgG drug substance

FILL–FINISH & QUALITY CONTROL

STAGE 04

01	FORMULATION	Prepare product-specific buffer and excipients.
02	STERILE FILTRATION	Filter appropriate liquid product before aseptic filling.
03	ASEPTIC FILLING	Fill and close vials or prefilled syringes in a controlled environment.
04	INSPECTION & TESTING	Verify identity, purity, potency, sterility and particulate quality.

Lyophilization is product-dependent, not required for every antibody medicine.

UPSTREAM PROCESSING EQUIPMENT

Objective: Grow healthy production cells under controlled conditions to maximize therapeutic protein production while maintaining the required product quality

01



Cryogenic Storage

Preserves Master Cell Bank (MCB) and Working Cell Bank (WCB) stocks.

02



Class II safety cabinet

Provides HEPA-filtered airflow for appropriate aseptic cell-handling operations.

03



Shaker incubator

Expands cells in flasks with orbital mixing for nutrient and oxygen transfer.

04

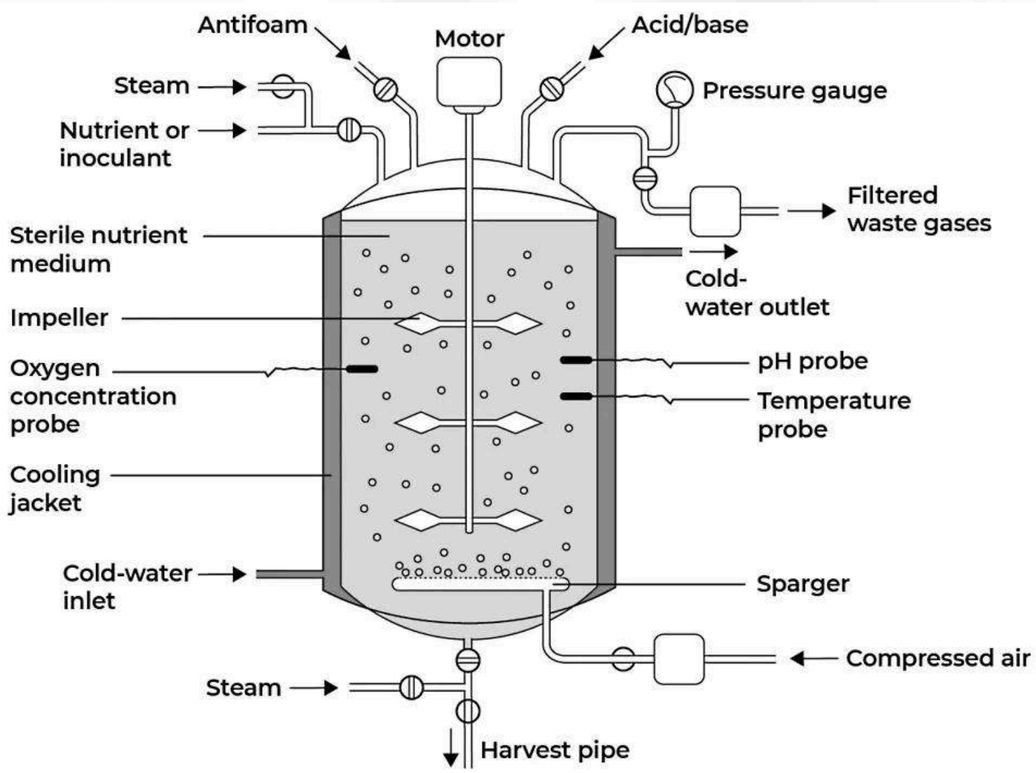


Seed bioreactor

Scales the inoculum through controlled culture volumes ahead of production.

BIOREACTOR





Production vessel

Controlled culture conditions

Sparger & gas mixer

O_2 supply and CO_2/pH control

Probes & controller

pH, DO, temperature, mass

Peristaltic pumps

Nutrient feed, pH dosing, transfer

UPSTREAM PROCESSING EQUIPMENT

05



Cell counter / viability analyzer

Measures total and viable cell densities, supporting the seed train, culture monitoring and harvest decisions.

HARVEST PROCESSING EQUIPMENT

Objective: Separate cellular solids from the liquid containing secreted antibody.

01





Disc-stack centrifuge

High centrifugal force removes bulk cells and debris from the harvest.

02



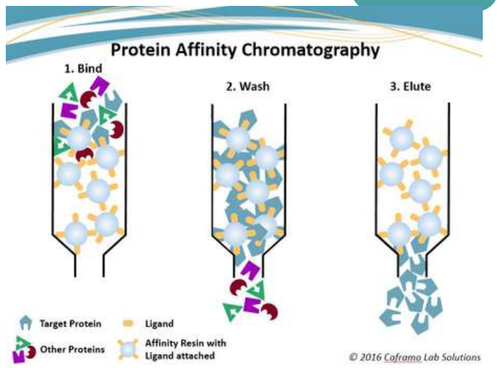
Ultracentrifuges

High-speed laboratory instruments designed to spin samples at extremely high rotational speeds, generating gravitational forces from 60,000 x g to over 1,000,000 x g

DOWNSTREAM PROCESSING EQUIPMENT

Objective: Isolate the therapeutic antibody from the clarified harvest, reduce host cell proteins, host cell DNA and product-related impurities, and establish the required viral safety profile

01



Protein G affinity column

Specialized chromatography tools used to capture, isolate, and purify immunoglobulins (IgGs) from biological samples like serum, and cell culture supernatants

02



Affinity Chromatography

Automatically separate, purify, and collect specific proteins from complex biological mixtures

03



UF / DF skid

Retains the therapeutic protein while permeating water and sufficiently small solutes. UF concentrates; DF exchanges buffer.

FILL-FINISH EQUIPMENT

Objective: Produce the specified drug-product composition, preserve product stability and deliver the medicine in a closed, sterile, correctly filled container

01



Formulation mixing tank

Gently blends the antibody with its defined buffer and excipients; controls pH and concentration.

02



Sterilizing-grade filter

Uses a validated filter—often nominal 0.2/0.22 µm—to remove retained microorganisms before aseptic fill.

03



Aseptic filling machine

Precisely dispenses sterile drug solution into vials, syringes or cartridges.

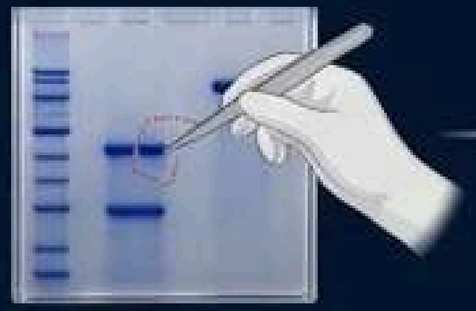
04



Lyophilizer

For suitable products: freezes and removes water under vacuum to support stability.

INTEGRATED LC-MS/MS WORKFLOW FOR IGG PEPTIDES



IN-GEL DIGESTION & ELUTION

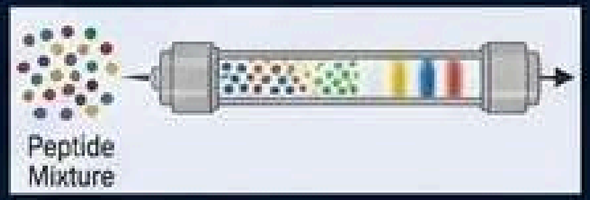
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2. Released peptides are eluted.





PEPTIDE SEPARATION BY NANOLC/HPLC

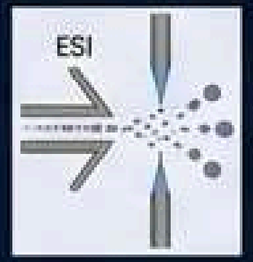
High-Resolution Separation: NanoLC/HPLC column separates complex peptide mixture based on hydrophobicity for sequential introduction to MS.



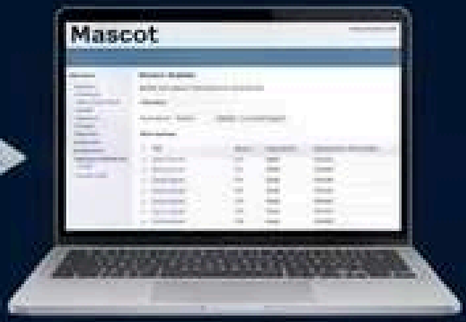


MASS SPECTROMETRY (MS/MS)

1. Peptides ionized (ESI) and introduced.
2. Precursor ions selected and fragmented.
3. Resulting fragment ions detected.







DATA PROCESSING & GLYCAN ANALYSIS

1. MASCOT database search identifies peptide sequences and protein identity.
2. Detailed analysis of N-glycosylation at specific sites (e.g., G0, G1, G2, Fc peptides).



- **Complementary Insight:** SDS-PAGE confirms molecular weight and purity (e.g., chains separated), while LC-MS/MS provides precise sequence-level identity and detailed post-translational modification analysis (e.g., glycosylation).

BEST SELLING BIOPHARMACEUTICALS IN CURRENT MARKET

Core Therapeutic Focus

Oncology, Metabolic, Immunology

Larana, Inc.

1. KEYTRUDA (PEMBROLIZUMAB)

Melanoma, Non-Small Cell Lung Cancer (NSCLC), Head & Neck Cancer, Triple-Negative Breast Cancer, and other solid tumors.



Merck & Co., Inc. is an American multinational pharmaceutical company headquartered in Rahway, New Jersey. The company does business as Merck Sharp & Dohme or MSD outside the United States and Canada. The company is ranked fifth on the list of largest biomedical companies by revenue.

"MERCK" trade name is used in U.S. and Canada (top); outside these countries "MSD" is used (bottom).

2. MOUNJARO / ZEPBOUND (TIRZEPATIDE)

Type 2 Diabetes Mellitus (Mounjaro) and Chronic Weight Management (Zepbound).

Key Growth Drivers: Industry-leading efficacy in glycemic control and significant weight loss, alongside rapid global uptake in anti-obesity markets.



3. Ozempic / Wegovy (Semaglutide)

Key Growth Drivers: High consumer demand for obesity management, expansion into cardiovascular and kidney disease risk reduction indications.

Novo Nordisk



4. Dupixent (Dupilumab)

Key Growth Drivers: Dominant market position in Type 2 inflammatory diseases and expanding approval into pediatric populations and COPD.



Sanofi & Regeneron

5. Skyrizi (Risankizumab)

Key Growth Drivers: High rates of skin clearance, long dosing intervals (every 12 weeks), and fast expansion in Inflammatory Bowel Disease (IBD) markets.



AbbVie

6. Darzalex (Daratumumab)

Key Growth Drivers:
Establishment as a standard backbone regimen in early-stage and frontline multiple myeloma treatment.



Johnson & Johnson (Janssen)

7. Humira (Adalimumab)

Key Market Dynamics: Historically the world's best-selling biologic; currently experiencing revenue shifts due to biosimilar entry in global markets.



AbbVie

8. Stelara (Ustekinumab)

- Key Growth Drivers: Strong efficacy profile in gastroenterology and dermatology, though facing incoming competition from biosimilars.



Johnson & Johnson

9. Eylea (Aflibercept)

Key Growth Drivers: High-dose formulations (Eylea HD) allowing extended treatment intervals for patient convenience.



Regeneron / Bayer

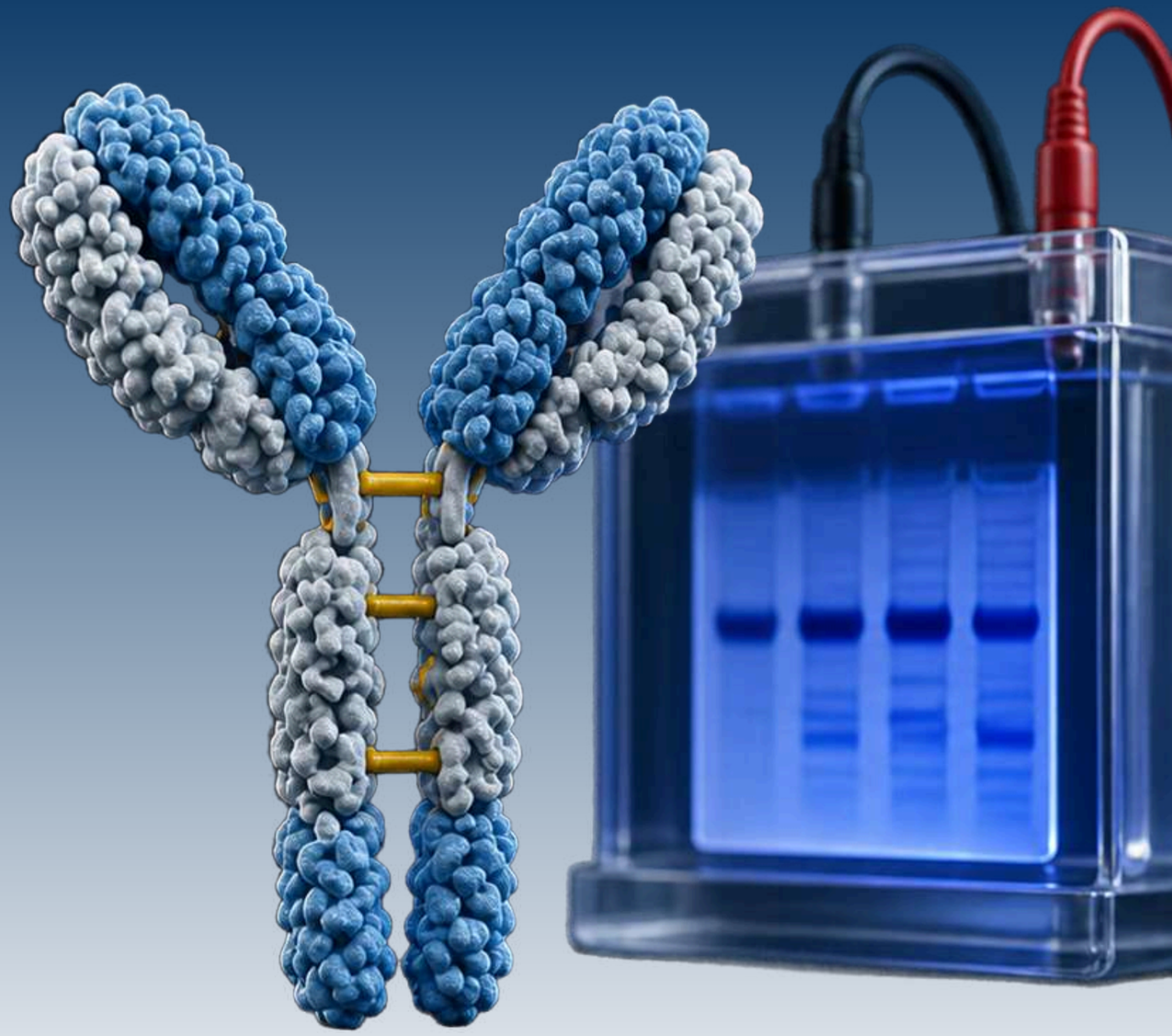
10. Biktarvy (Bictagravir / Emtricitabine / Tenofovir Alafenamide)

Key Growth Drivers: Positioned as the single-tablet treatment regimen for treatment-naïve and virologically suppressed patients.



Gilead Sciences

How Are IgGs Separated by SDS-PAGE?

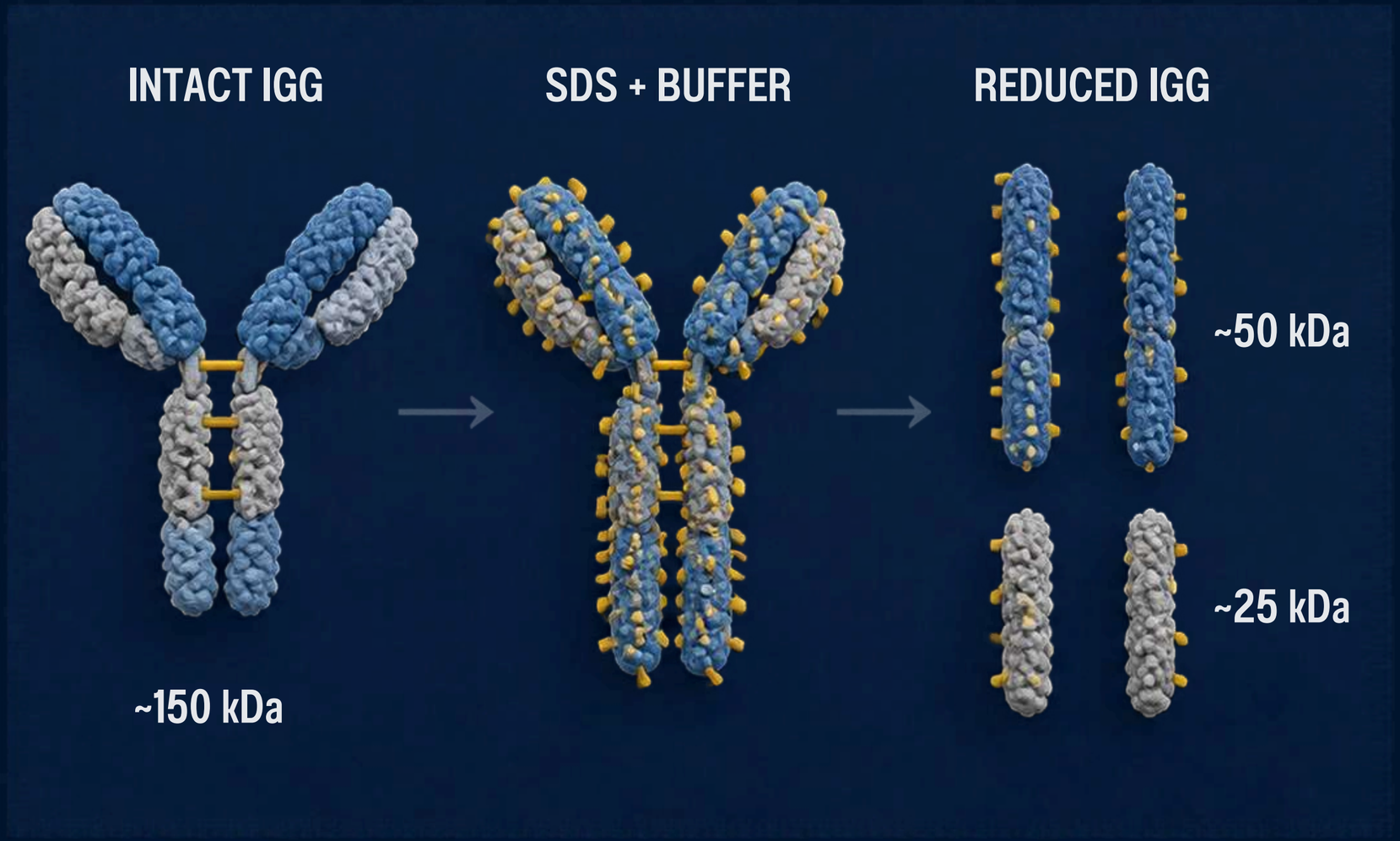


SAMPLE PREPARATION FOR SDS-PAGE

Denaturation & Reduction: IgG is treated with SDS buffer and reducing agents (\beta-ME or DTT) to break disulfide bonds.

Chain Separation: Separates intact IgG (~150 kDa) into Heavy Chains (~50 kDa) and Light Chains (~25 kDa).

Electrophoresis Mechanism: Polyacrylamide gel acts as a molecular sieve–smaller proteins move faster and travel farther under an electric field.



RESULTS AND DETECTION OF IGG BANDS BY SDS-PAGE

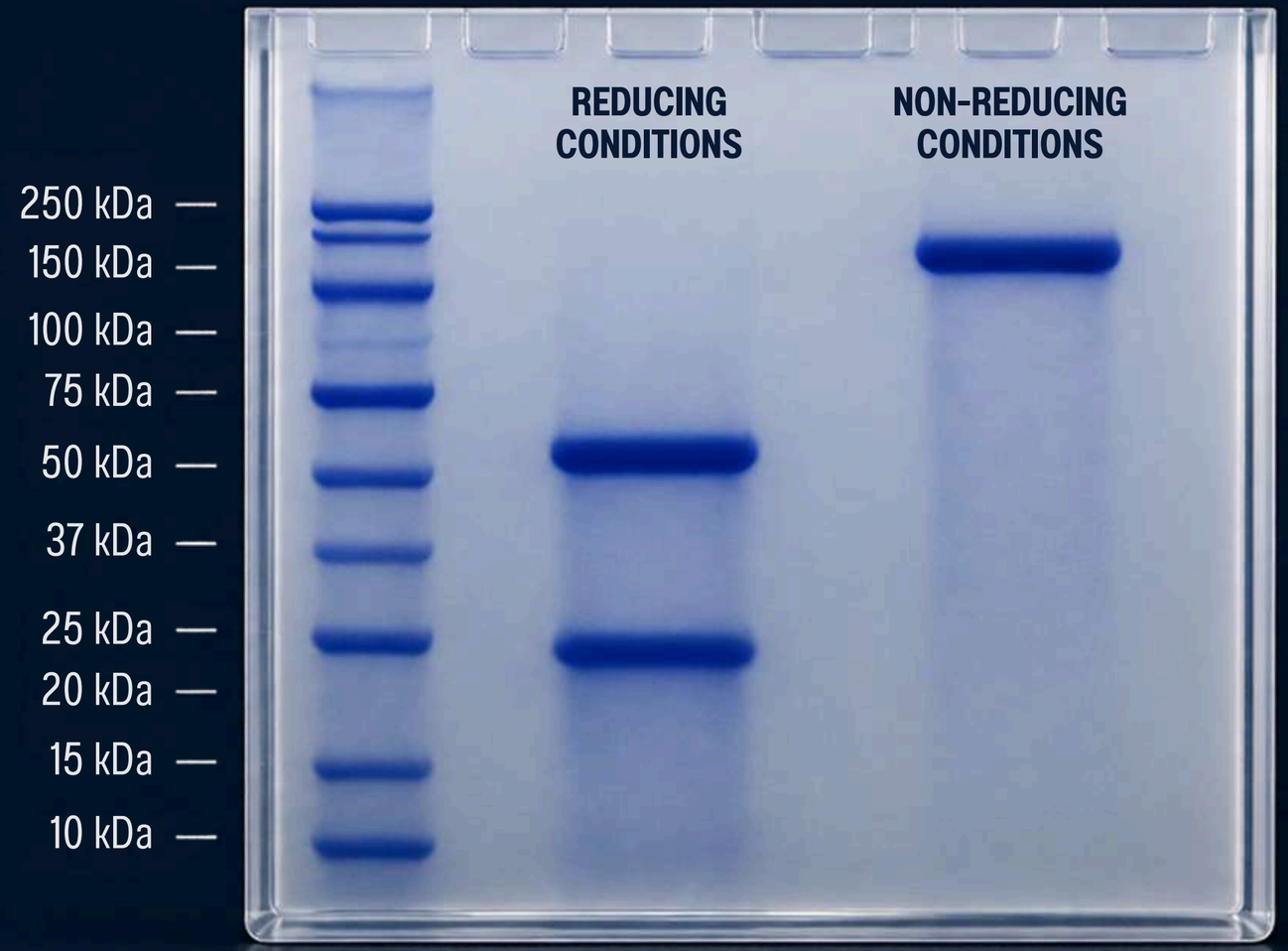
Stained with
Coomassie Brilliant Blue
or silver stain

REDUCING CONDITIONS

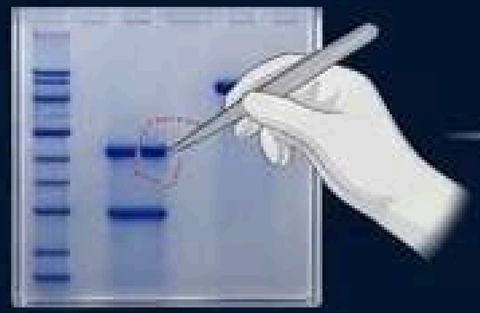
Two bands are observed
at ~50 kDa and ~25 kDa

NON-REDUCING CONDITIONS

Intact IgG migrates
at ~150 kDa

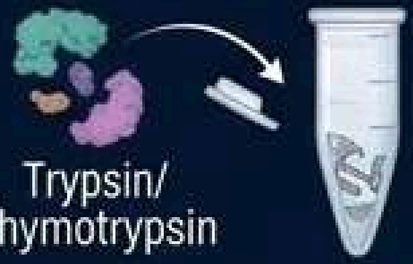


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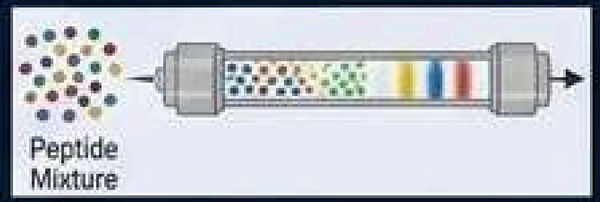
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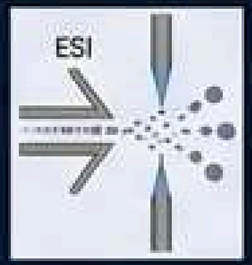
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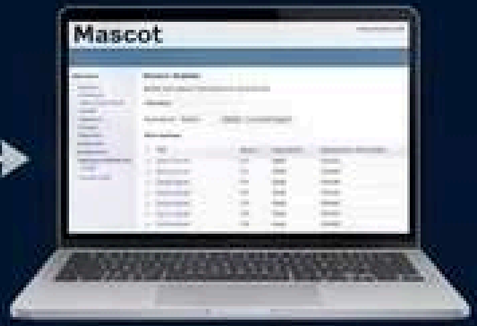


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