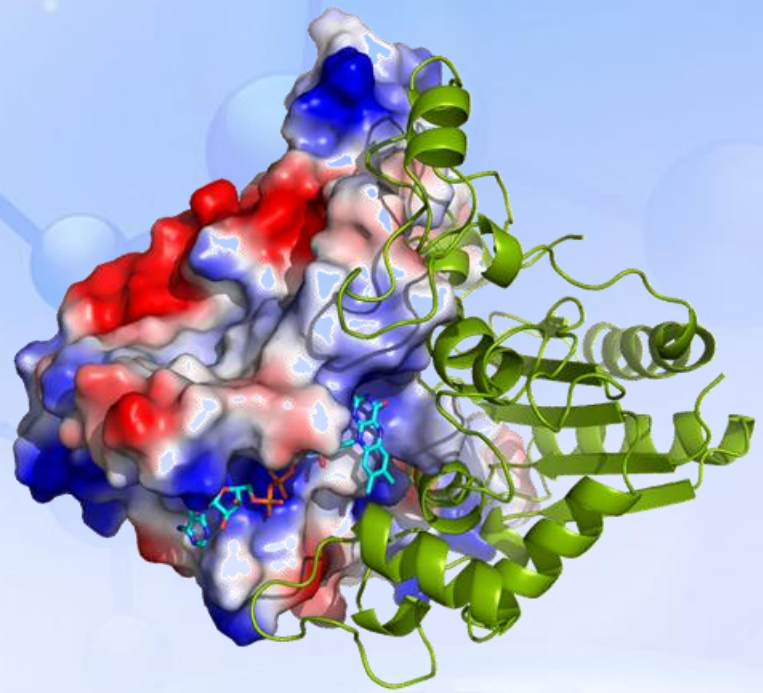
BIORTUS

A Protein-Focused Partner

Scientists for Scientists: Protein Purification At Biortus



February, 2025

Streamlined for Fast Turnaround

BIORTUS

Guided Design from the Beginning

- Published Literature & Structures
- Structure Prediction Tools

An Extension of Your Lab

- Membrane, Soluble, & Refolded Proteins
- Standard & Specialty Columns

4-8 weeks

Evaluation & Design

Expression

Purification

Quality Control

Construct & Condition Screening

- High-throughput parallel screening
- Shakers, Fermenters, WAVE
- *E. coli,* Insect Cell, Mammalian, Silkworm, etc.

Soluble, Consistent, Functional

- Standard QC: SDS-PAGE, LC-MS, aSEC
- Custom QC: nanoDSF, SPR, Negative Stain, etc.

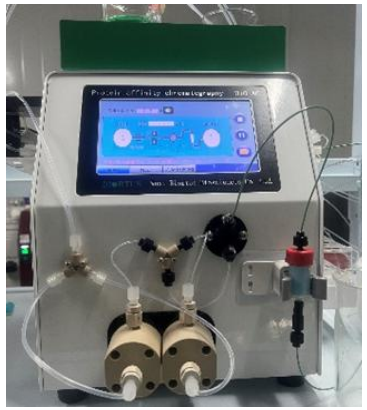
Our facilities and instruments available

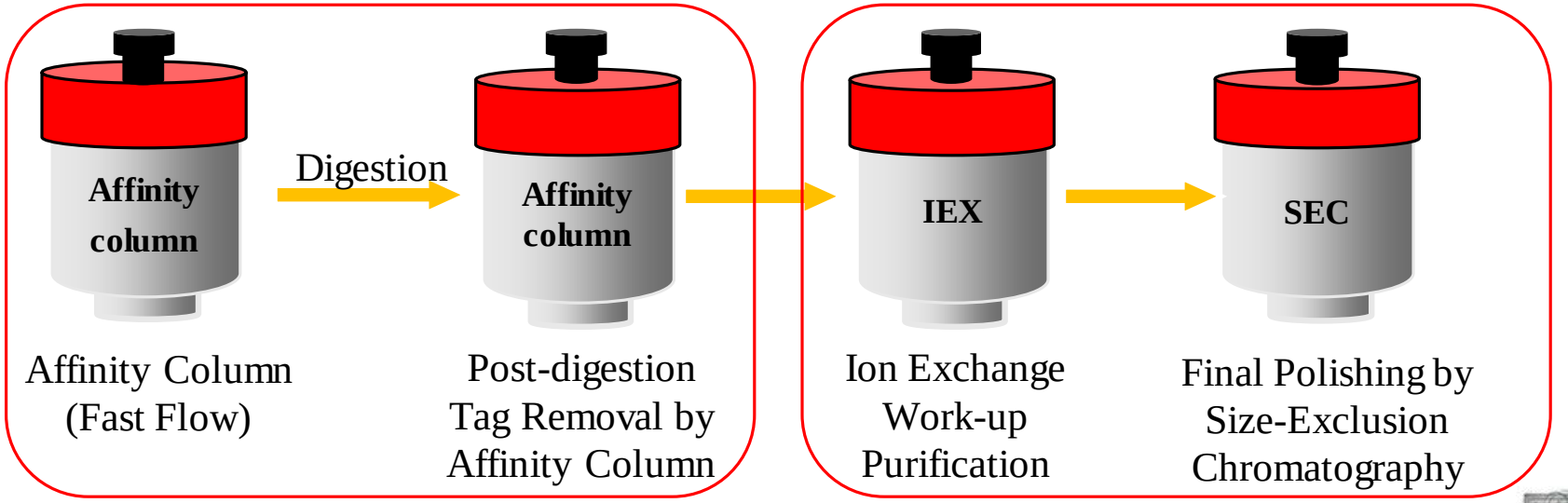
- >50 AKTAs
- >250 employees in protein expression and purification department
- Dedicated membrane protein purification team
- Over 200 batches of purified protein per week
- Endotoxin removal available
- Standard columns and specialty columns available
  - Nickel (NTA, Excel, etc.), GST, MBP, Flag, Protein A/G, IEX, SEC, and more.

AKTAs At Biortus (>50 Instruments In Total)

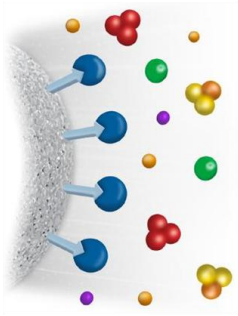
BIORTUS 佰翱得

Core Steps in the Purification Workflow



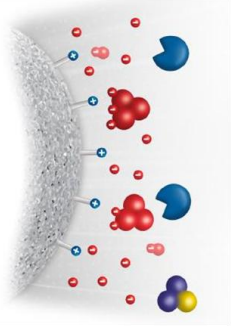


Affinity chromatography (AC)

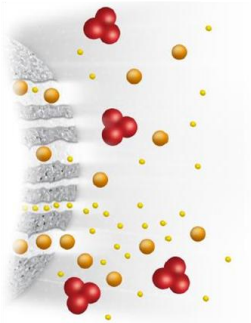


- Bind – elute principle
- Requires specific elution conditions
- Concentrating effect

Ion exchange chromatography (IEX)



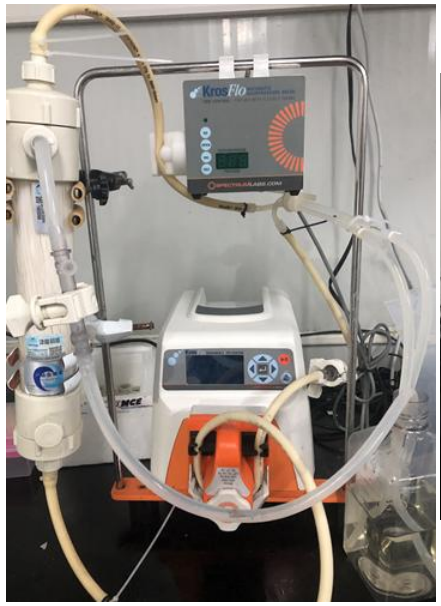
Size exclusion chromatography (SEC)



- Diffusion – no binding
- Any elution conditions
- Diluting effect

Protein Preparation Platform-Purification (Cell Lysis)

For cell pellet



For culture medium

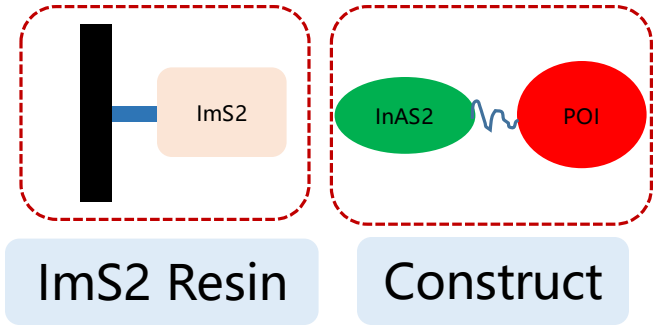
Primary Purification: Affinity Chromatography

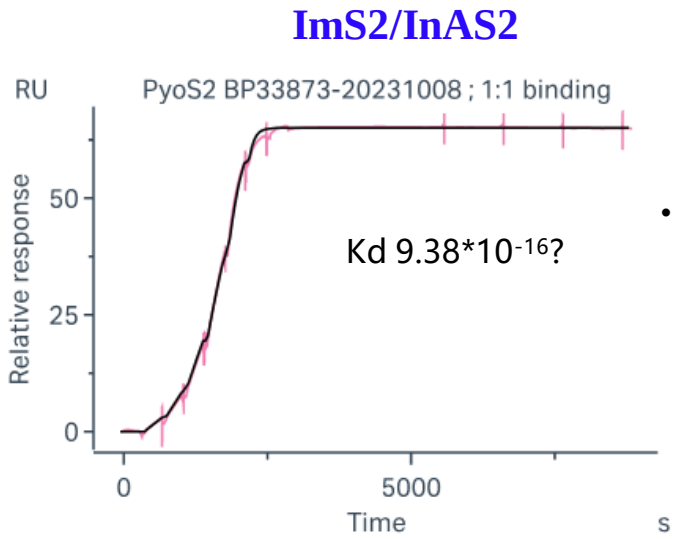
BIORTUS

Tag	Sequence	Residues	Affinity K_D (M)	Loading NaCl (M)	Capacity (mg/ml)	Affinity beads
His	HHHHHH	6	$10^{-8/-9}$	---	20-40	His FF
Strep	WRHPQFGG	8	10^{-6}	---	---	Strep-Tactin
StrepII	WSHPQFEK	8	10^{-9}	---	10-15	Strep-Tactin XT
Flag	DYKDDDK	8	$10^{-8/-9}$	< 0.2	0.5-1	Anti-Flag G1
HA	YPYDVPDYA	9	10^{-9}	< 0.2	---	4B2 antibody
ALFA	SRLEEELRRRLTE	13	10^{-11}	< 0.2	---	ALFA nanobody
Twin StrepII	SHPQFEK-(GGGS)2-GGSAWSHPQF	28	10^{-12}	---	10-15	Strep-Tactin XT
CL7	TFWEEVSKDPELSKQFSRNNNDRN	130	$10^{-14/-17}$	---	15-20	IM7
GST	VLDIRYGVSRIAYSKDFETLKVDFL	218	$10^{-6/-7}$	< 0.2	10	GST FF
MBP	KYDIKDVGVDNAGAKAGLTFLVD	370	10^{-6}	< 0.2	6-10	MBP FF
SpyTag	AHIVMVDAYKPTK	13	Covalent	---	---	Catcher-Protein
Biotinylated-Avi	Biotin+GLNDIFEAQKIEWHE	15	$10^{-12/-13}$	---	10-15	Strep-Tactin XT
HiBiT	MVSGWRLFKKIS	11	10^{-9}	---	---	LgBiT

- Preferred construct design: **His-StrepII-TEV-GG-POI,** Screened by FSEC+His Lite methods.

Biortus: Affinity Chromatography-ImS2/InAS2





- Both the affinities of Im7/CL7 and ImS2/InAS2 are beyond the limit of the S200 SPR instrument, we termed as "Infinite Affinity (InA)".

Beads	Binding Affinity(M)	Purity	Binding Capacity (mg/mL)	Recycle
His	10^{-8}-10^{-9}	>80%	40	5
Flag	10^{-8}-10^{-9}	>90%	1	2
GST	10^{-6}-10^{-7}	>90%	5	5
StrepII	**10^{-12}**	**>90%**	**14**	**10**
ImS2	**Very high**	**>90%**	**70**	**10-100**
Im7	**Very high**	**>90%**	**40-60**	**10-100**

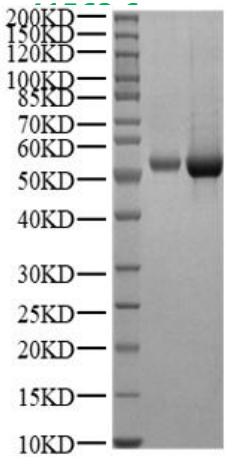
❑ Features:

- can be attached to either N- or C-terminus of POI
- can be expressed in *E.coli*, insect cell and mammalian systems
- can be used for either cellular or secretion expression

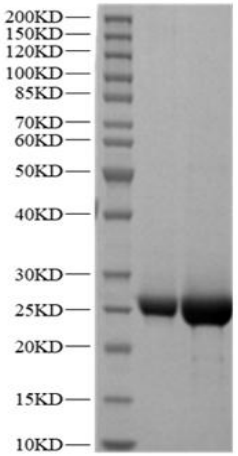
Biortus: One Step Infinite Affinity (InA) Purification

E.coli system

37641,

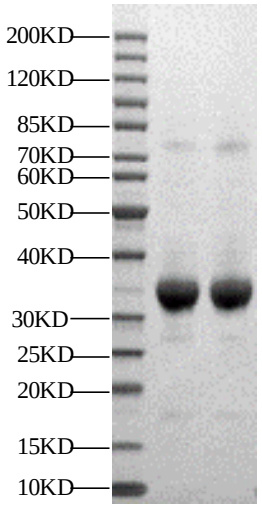


37664, 27217.8



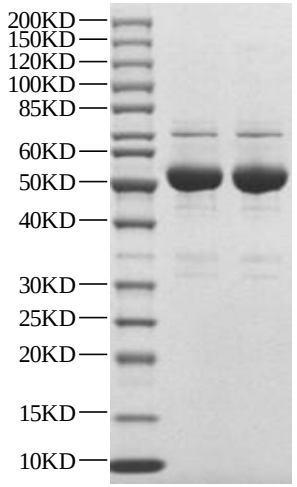
Insect, intracellular

39787, 37535.9



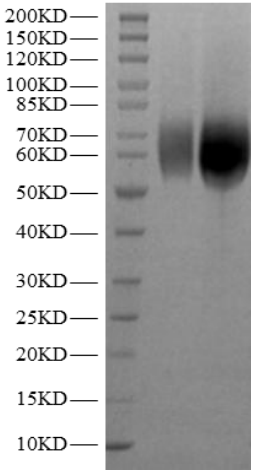
Mammalian, intracellular

38352, 48375.2

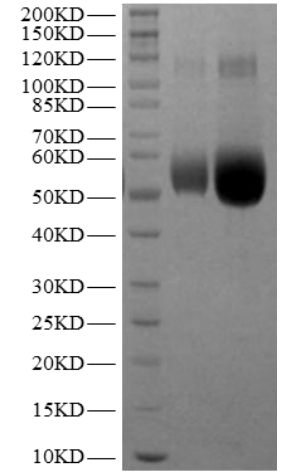


Mammalian, secreted

39782, 38779.9



39777, 39675.3

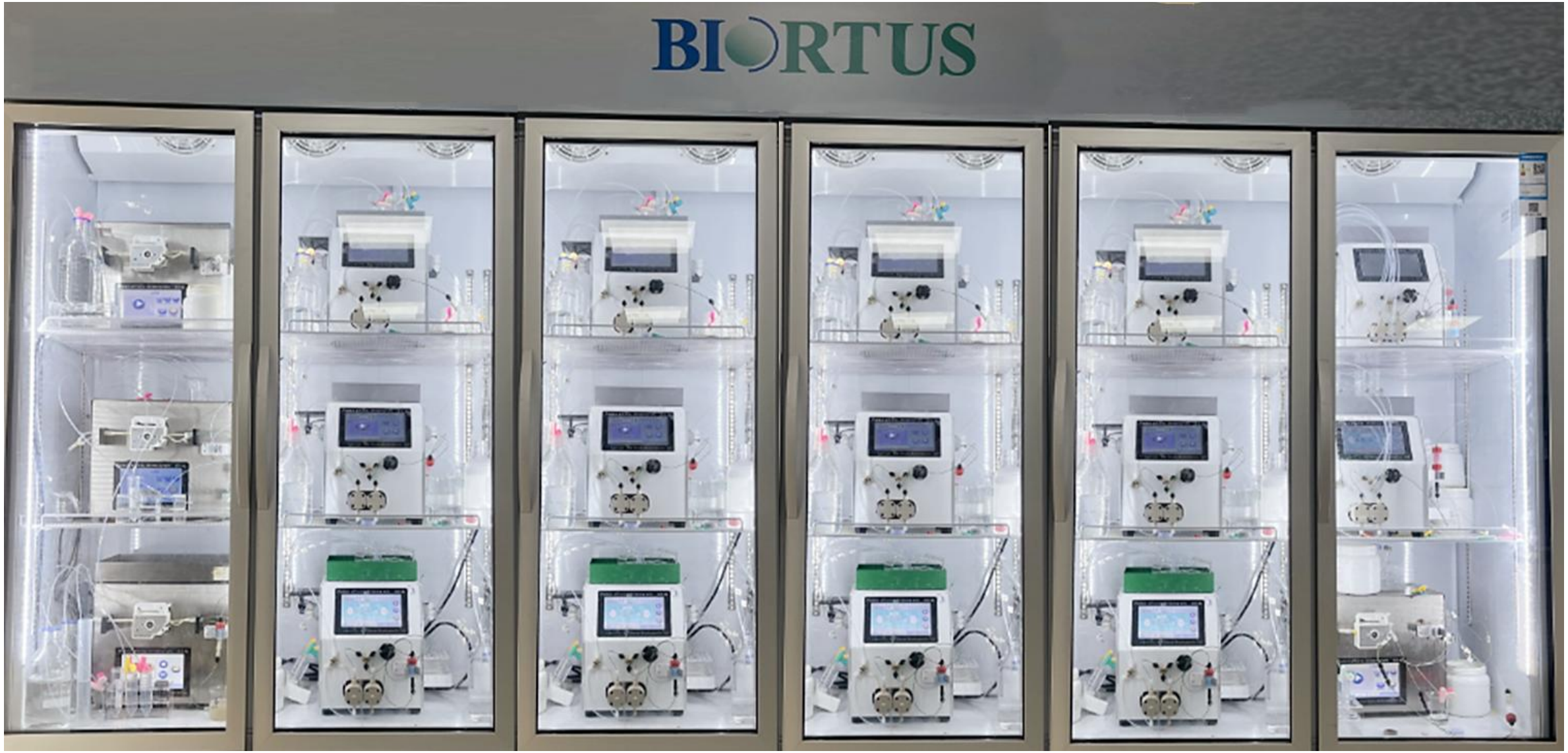


Introduction Self-developed Bio-AC™

Bio-AC™ is a fast protein affinity purification system that takes up very little laboratory space and can automate sample affinity chromatography with a simple program setup. It can help experimentalists reduce the probability of human error and the time of experimental operation.

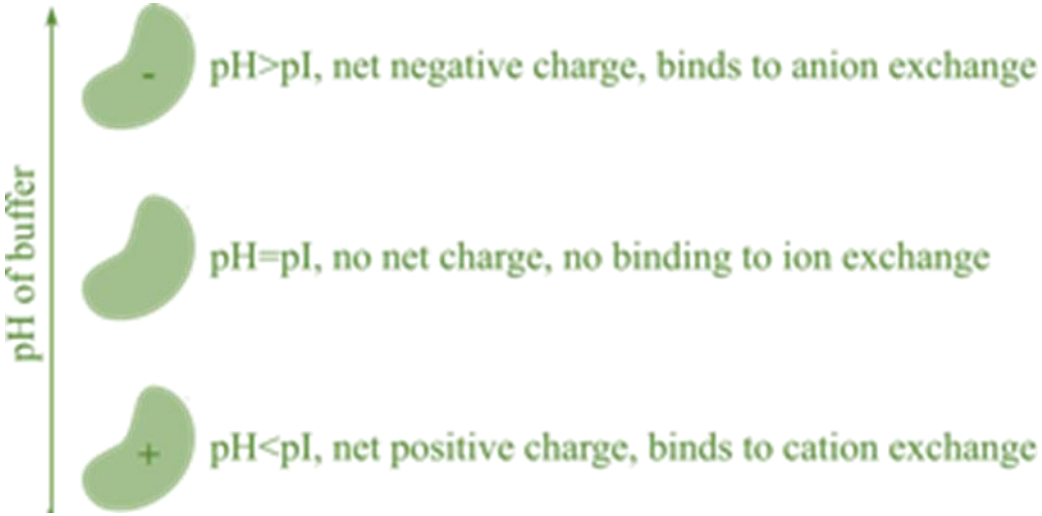
Comparison item	Bio-AC
Pump	Double plunger pump
Air sensor	+(Ultrasonic wave)
UV detector	-
Collector	Eight channel outlet valve
Control system	Touch screen
Column value	+(2 column position)
Pressure detector	+
Flow rate（ml/min)	0.1-20
Max pressure（MPa)	0.7
Price	Low price

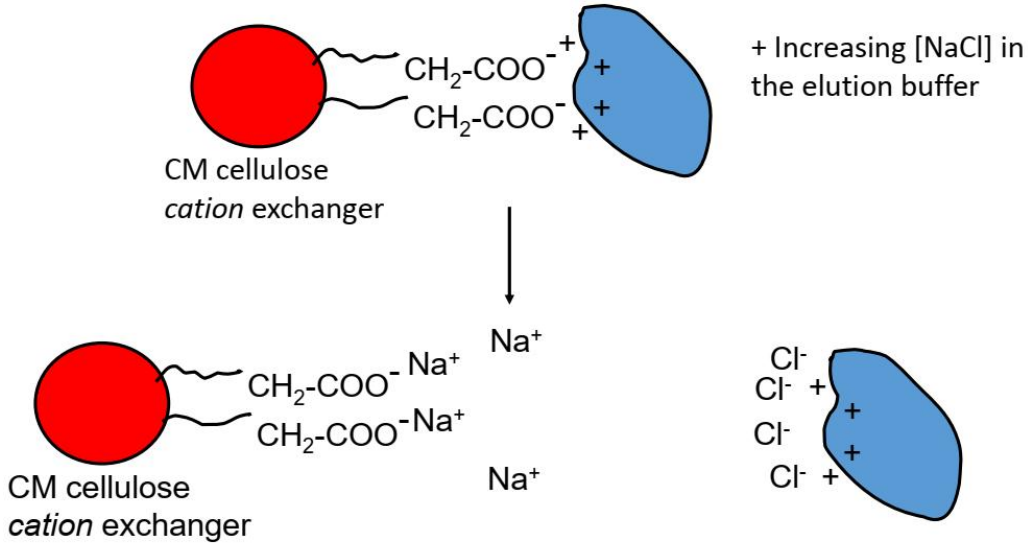
Bio-AC™ at Biortus



Polishing Purification: Ion Exchange Chromatography (IEC)

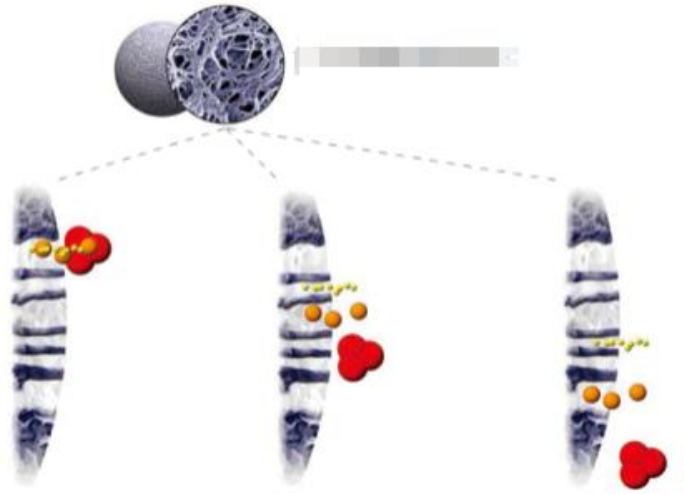
Group	Abbreviation	Exchange Type	Structure
sulphonate	S	strong cation	-CH2SO3-
quaternary amine	Q	strong anion	-CH2N+(CH3)3

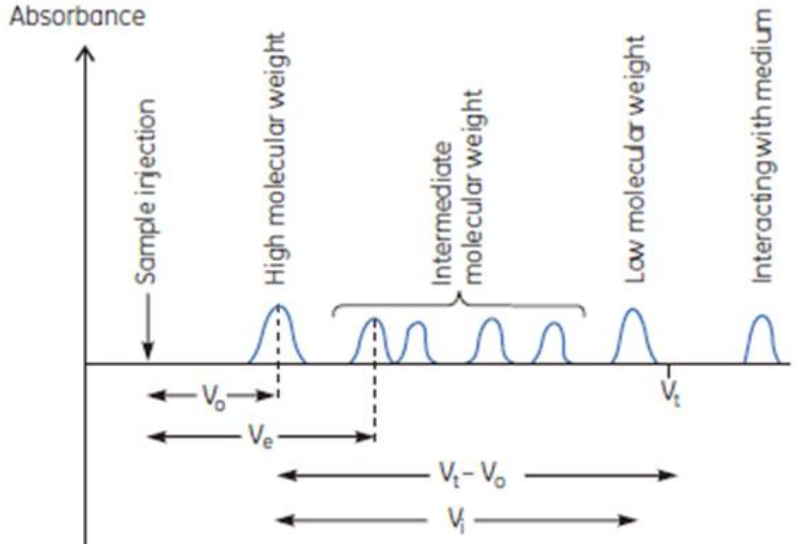




IEX	**Mono Q 10/100 GL, Mono S 10/100 GL**	**8ml**
	Resource Q, Resource S	**6ml**
	Source 15Q 4.6/100 PE, Source 15S 4.6/100 PE	**1.7ml**
	HiTrap Q HP / FF; HiTrap SP HP / FF	**1ml, 5ml**

Polishing Purification: Size-Exclusion Chromatography (SEC)





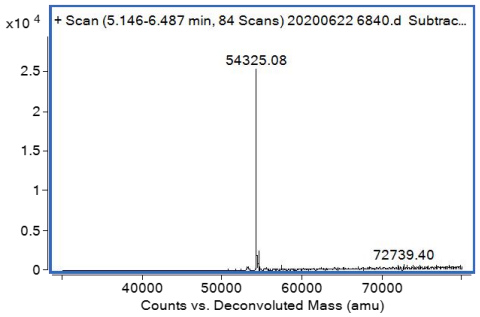
Column	Specification	Separation range	Sampling volume	Sampling volume	Flow rate	Pressure
Superdex 200 Increase 5/150 GL	3ml	QC, or small volume	<50μl		0.3ml/min	
Superdex 200 Increase 10/300 GL	24ml	10~600KD	<0.5ml	<10mg	0.5ml/min	3.5MPa
Superdex 75 Increase 10/300 GL	24ml	3~70KD	<0.5ml	<10mg	0.5ml/min	3.5MPa
Superose 6 Increase 10/300 GL	24ml	5~5000KD	<0.5ml	<10mg	0.5ml/min	3.5MPa
HiLoad 16/600 Superdex 200 pg	120ml	10~600KD	<5ml	<60mg	1.0ml/min	0.5MPa
HiLoad 16/600 Superdex 75 pg	120ml	3~70KD	<5ml	<60mg	1.0ml/min	0.5MPa
HiLoad 16/600 Superdex 30 pg	120ml	3~30KD	<5ml	<60mg	1.0ml/min	0.5MPa
HiLoad 26/600 Superdex 200 pg	320ml	10~600KD	<13ml	<120mg	1.5ml/min	0.5MPa
HiLoad 26/600 Superdex 75 pg	320ml	3~70KD	<13ml	<120mg	1.5ml/min	0.5MPa

Protein Preparation platform-Reaction of modification

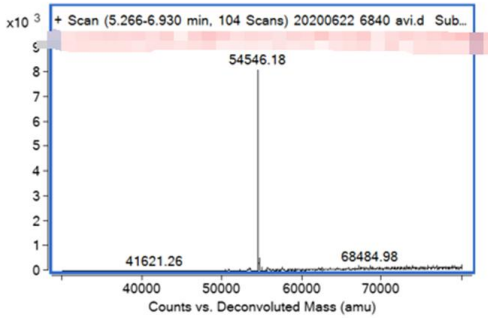
➢ Biotinylation

Commonly used enzymes: BirA

Before biotinylation



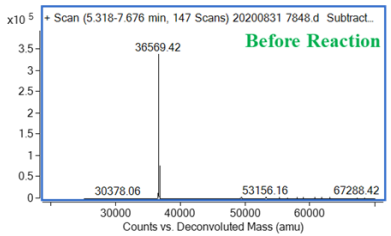
After biotinylation

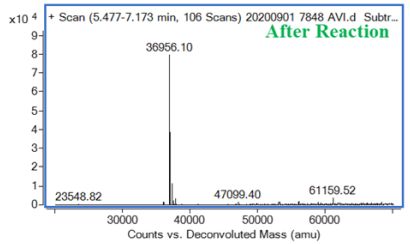


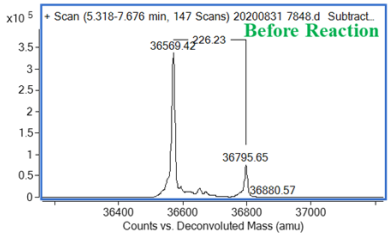
➢ Phosphorylation

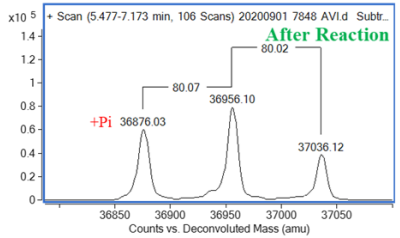
Commonly used enzymes: CAK， SRC

Result:





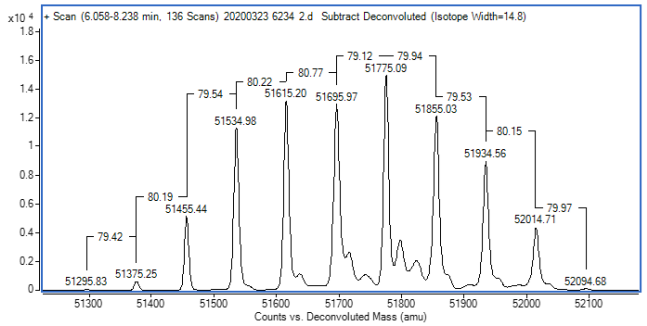




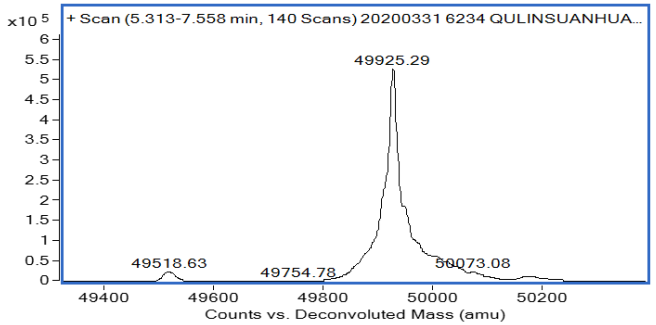
➢ Dephosphorylation

Commonly used enzymes: LamdaPP

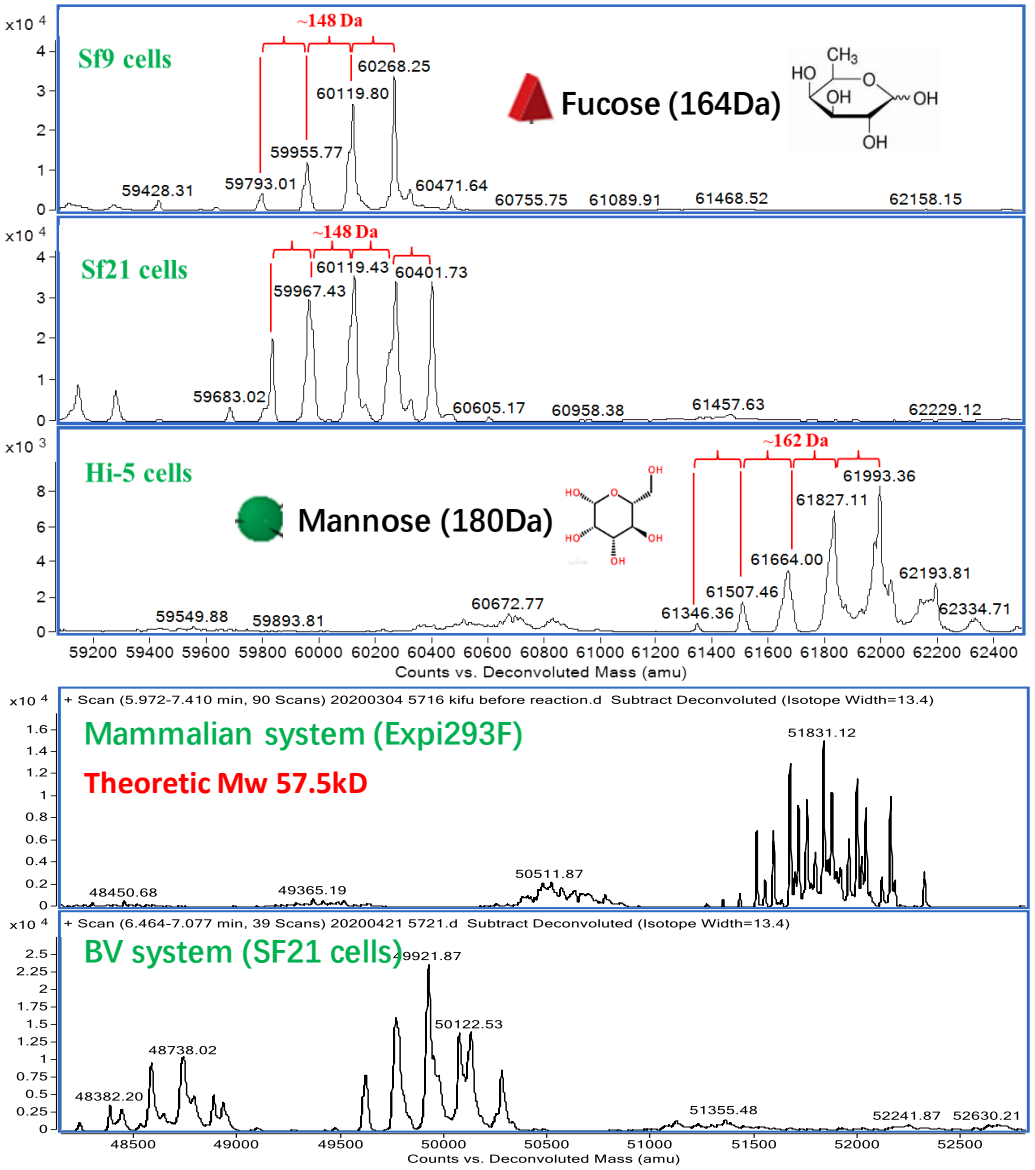
Before Dephosphorylation

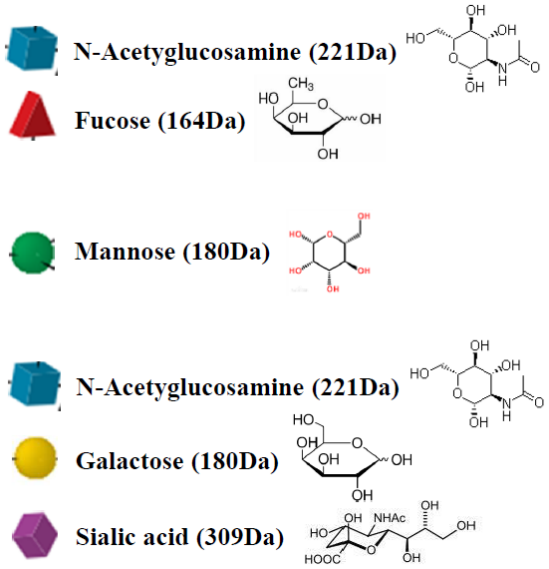


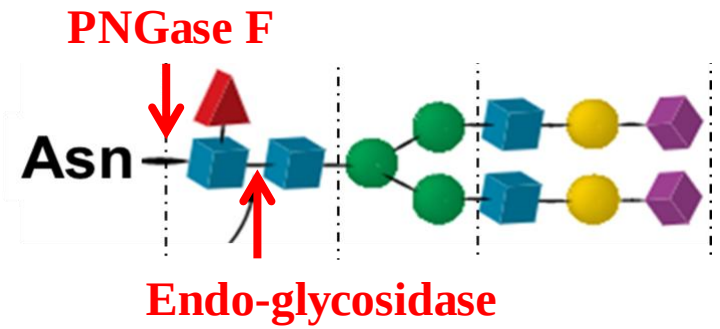
After Dephosphorylation



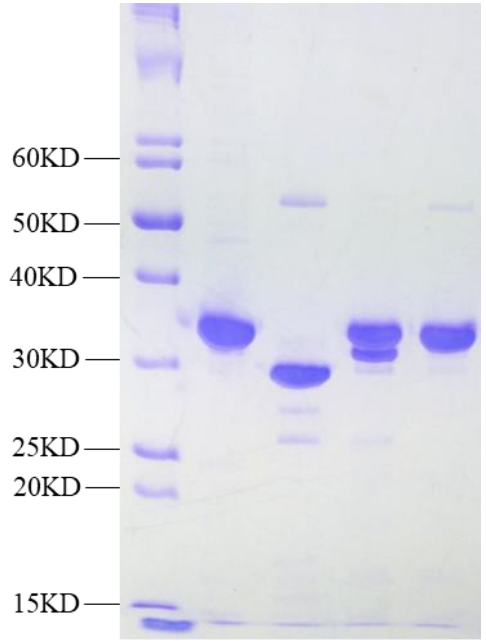
Glycosylation in insect cell and mammalian cell expression







Theoretic Mw: ~35kD



M = Marker
Lane 1 = before reaction
Lane 2 = Endo F1-overnight
Lane 3 = Engo F2-overnight
Lane 4 = Endo F3-overnight

Convenient and Specific Multi-functional labeling

BIORTUS

(Re)Labeling with Sortase

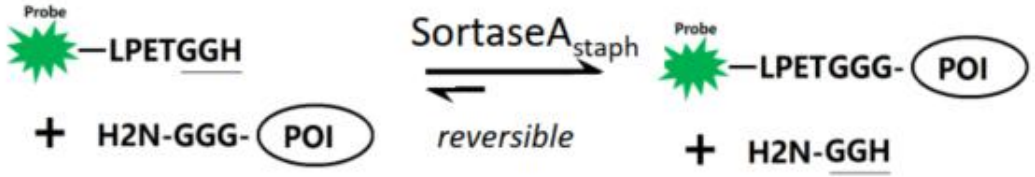
- Post-purification Tag addition
- Convenient & Specific Labeling with High-Efficiency
- Conjugate Dyes

For your pipeline

- Time saved: No need to start over for new affinity tags
- Common applications: Biotinylation for SPR, Fluorescent labeling

All constructs at Biortus integrated

- His-StrepII-TEV-GG-POI → GGG-POI
- Natural Substrate for Sortase



Example: Fluorescent labeling with FAM & Alexa647

Item	No.	Amount of G	MW(Da)	Peptide	Label efficiency	Time in RT	Time in 4°C
1	6076	G	33851.39	FAM-Ahx-LPETG(2-hydroxyacetic acid)-GH	~100%	2h	2~4h
2	8168	GGG	25573.50	FAM-Ahx-LPETG(2-hydroxyacetic acid)-GH	~100%	1h	1~2h
3	8198	GGG	17948.03	FAM-Ahx-LPETG(2-hydroxyacetic acid)-GH	~100%	1h	1~2h
4	7109	G	17711.75	FAM-Ahx-LPETG(2-hydroxyacetic acid)-GH	~10%	4h	4h

Quality Control is Standard Practice

BIORTUS

Standard QC Package

SDS-PAGE

Purity
Stoichiometry

LC- MS

MW verification
Protein
Validation
PTMs

Analytical SEC

Solubility
Multimerization
Complex
Integrity

Custom QC Options

- Functional biochemical & biophysical assays
- SEC-MALS: oligomerization and solubility
- De-glycosylation
- Complex formation (gel-shift)
- Neg-stain EM
- Mass photometry
- DSF: Tm determination/stability

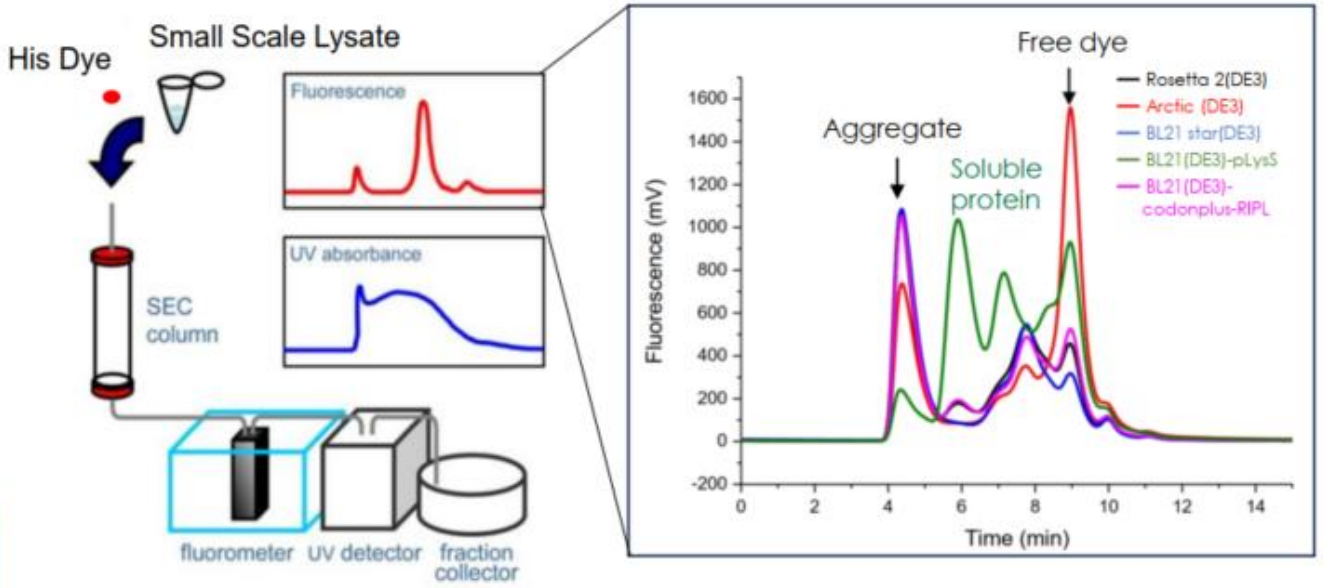
FSEC facilitates high-throughput optimization

Through the Bottleneck: FSEC

- In parallel testing and monitoring
- Input – Small Scale Expression Lysate
- Visualization: His-Dye, GFP, Conjugated Antibody
- Up to 100 conditions or constructs /day /instrument (5 Total)

For your pipeline

- FL vs. Truncations vs. Mutations
- Expression Host System & Conditions
- Buffer & Solubility Screening
- Complex Formation

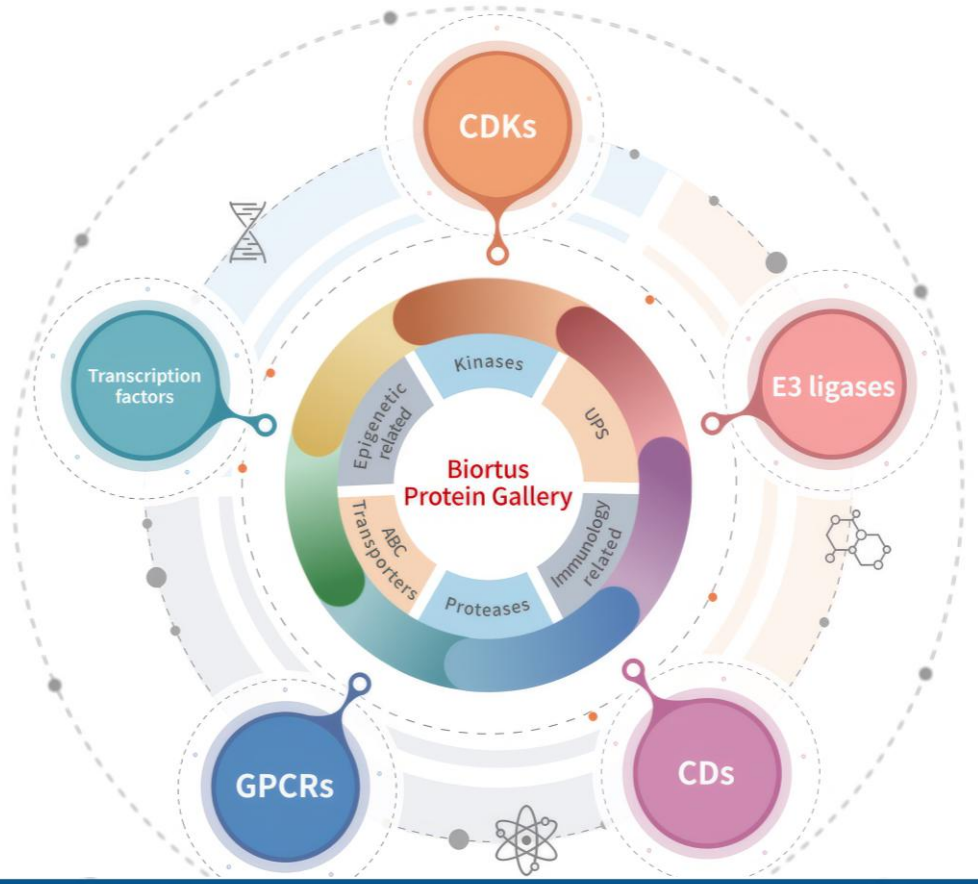


Biortus: Your Trusted Partner

BIORTUS

Specialize in Drug Targets

- Current inventory at over 2500 and expanding
- Focus on Drug Targets (E1, E2, E3, DUB, Ras, CDK, GPCR, SLC, etc.)
- High standards for QC
- >600 proteins, 17 assay kits are available on VWR and Chem-space and more products are coming.



BIORTUS
Protein Focused Services for Drug Discovery
From Gene to Structure

avantor™ delivered by VWR™ | VWR.COM

Protein catalog is updated every month with more proteins included in the list.
Please check with us for current information.

Biortus: Your Trusted Partner

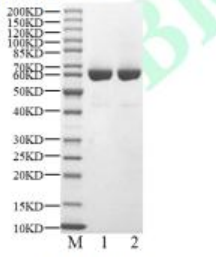
Delivery standard

STAT3

Category No.	**BP39735-00A**
Lot #	0001
Species	*Homo sapiens*
Uniprot ID/NCBI	P40763
AA sequence	G127-I722
Tags & Cleavage sites	No tag
Expression System	*E.coli*
92.6	67.9 KDa
Concentration	2.07 mg/ml **
Storage Temperature	-80°C
Storage Buffer	50 mM HEPES (pH7.5), 200 mM NaCl, 20% glycerol, 1 mMDTT
Notes	**Please use rapid thawing with running water to thaw the protein samples.** For complete recovery, mix well and spin before use. Product must not be store in diluted solutions. Aliquots below 10μl are not advisable. **Avoid repetitive freeze-thaw cycles!**

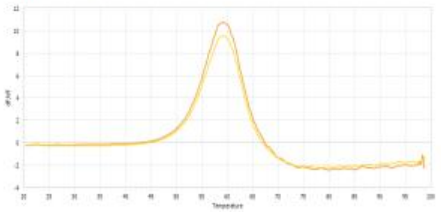
**Measured using UV absorption

SDS-PAGE



Lane M: Marker
Lane 1: 2 μg, reduced
Lane 2: 2 μg, non-reduced

Tm =59.16 °C

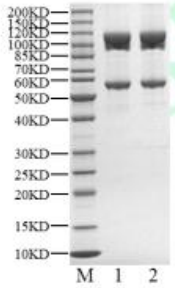


CRBN/DDB1

Category No.	**BP12360-01A**
Lot #	0001
Species	*Homo sapiens*
Uniprot ID/NCBI	Q96SW2/Q16531
AA sequence	M1-L442 end(CRBN) M1-H1140 end(DDB1)
Tags & Cleavage sites	N-His-TEV-Avi (CRBN) No tag (DDB1)
Expression System	Insect cell
MW	55.3 KDa/126.9 KDa
Concentration	1.71 mg/ml **
Storage Temperature	-80°C
Storage Buffer	50 mM Tris-HCl (pH7.5), 200 mM NaCl, 20% glycerol, 1 mM DTT
Notes	For complete recovery, mix well and spin before use. Product must not be store in diluted solutions. Aliquots below 10μl are not advisable. **Avoid repetitive freeze-thaw cycles!**

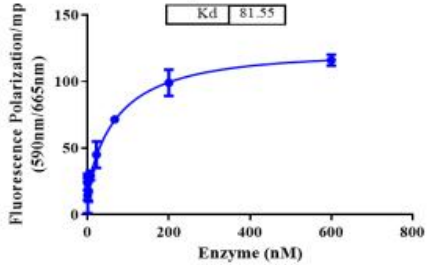
**Measured using UV absorption

SDS-PAGE



Lane M: Marker
Lane 1: 2 μg, reduced
Lane 2: 2 μg, non-reduced

Kd Titration assay



CDK2/Cyclin A2

Category No.	**BP469A2-21A**
Lot #	0002
Species	*Homo sapiens*
Uniprot ID/NCBI	P24941/P20248
AA sequence	M1-L298(CDK2) E174-L432(Cyclin A2)
Tags & Cleavage sites	N-GST-3C(CDK2) N-6His-TEV(Cyclin A2)
Expression System	Insect cell
MW	60.4 KDa/32.8 KDa
Concentration	2.29 mg/ml **
Storage Temperature	-80°C
Storage Buffer	20 mM HEPES (pH 7.5), 200 mM NaCl, 5% glycerol, 1 mM DTT.
Notes	**Please use rapid thawing with running water to thaw the protein samples.** For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions. Aliquots below 10μl are not advisable. **Avoid repetitive freeze-thaw cycles!**

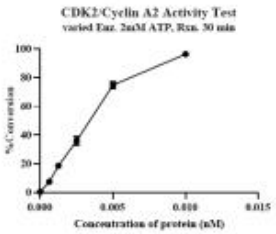
**Measured using UV absorption

SDS-PAGE

Lane M: Marker
Lane 1: 2 μg, reduced

Caliper assay

ATP-Km=65 μM



Thanks!

Biortus Biosciences Co., Ltd.
https://en.biortus.bio/

For custom: info@biortus.bio

For off-the-shelf: order@biortus.bio