XBio, Focus on Protein

清泓生物技术（上海）有限公司
www.xbioservice.com

Platform for active protein production

- **Mammalian expression for correct protein phosphorylation and modification**
- **Optimized procedure for protein expression in insect cells from Bacmid to Virus**
- **Soluble protein expression in *E.Coli***
- **Delicate process for protein purification**
- **Unique experience for assay development based on protein activity, protein and protein interaction**

清泓生物技术（上海）有限公司
www.xbioservice.com

Capacity

- Mammalian cells: Pilot study of 50 constructs in parallel, 5-25 mg per batch
- Insect cells: Pilot study of 50 constructs in parallel, 10-50 mg per batch
- *E.Coli*: Pilot study of 50 constructs in parallel, 10-100 mg per batch
- The capacity could be expanded upon request

清泓生物技术（上海）有限公司
www.xbioservice.com

Case study:
Gene to Structure of Kinase S

清泓生物技术（上海）有限公司
www.xbioservice.com

Expression of recombinant Protein S

Recombinant plasmids	Expressed protein	Co-transformed plasmid	E.Coli Strain	Medium	Induced Conditions
pET28B-TEV-Protein S2	His tag-thrombin-TEV-Protein S2	pCDFDuet1-YopH	BL21(DE3)	TB	18 ℃, 14hrs, 0.2mM IPTG

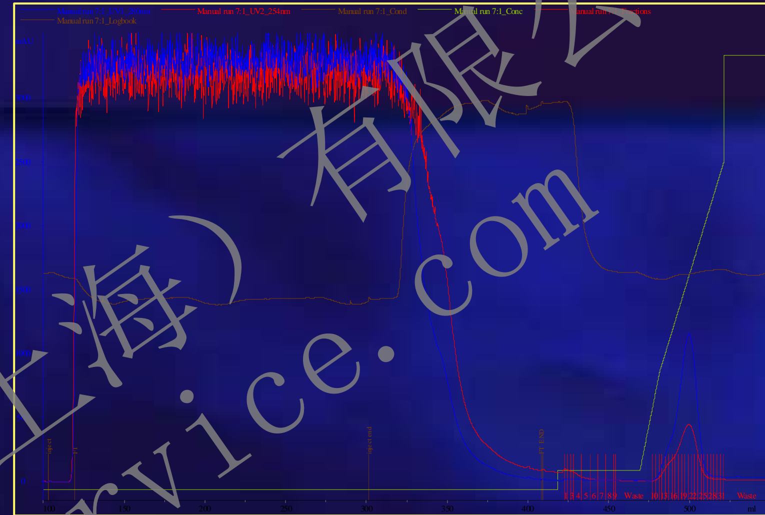
Protein	MW (Da)	Theoretical PI	Amino acids
His tag-thrombin-TEV-Protein S2	35495.856	6.075	311
Protein S2	32349.409	5.315	283

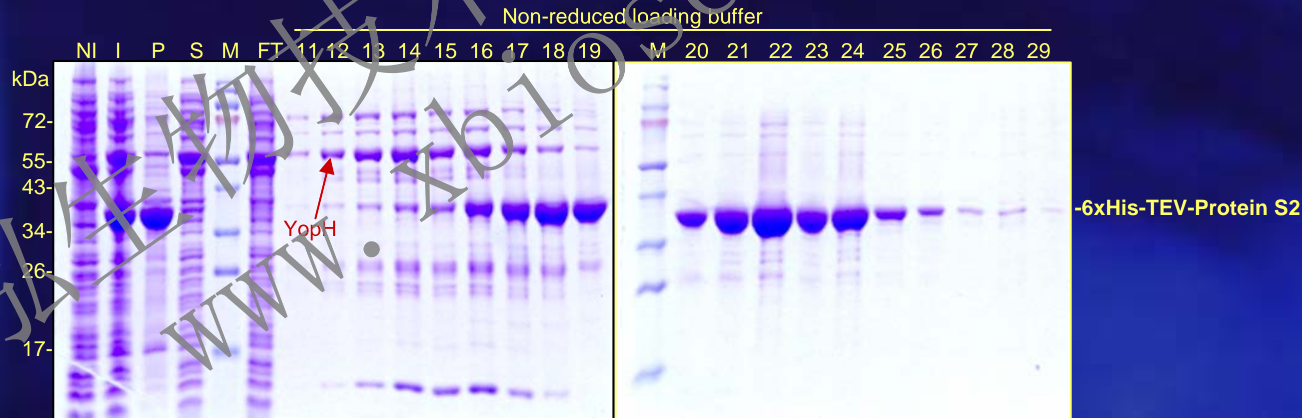
	OD600
Plasmids	pET28b-TEV-Protein S2
	pCDFDuet1-YopH
Seed-13:10	2.0211
Theoretical-13:10	0.0396
14:10	0.2060
14:41	0.4208
15:11	0.8290 Lower the temperature to 18℃
16:10	1.5227 Add IPTG
2nd day, 8:10	16.4340 Collect the culture.

清泓生物技术（上海）有限公司
www.xbioservice.com

Purification step one: Ni-Column

Sample	1.5L cell lysate supernatant
Column	HisTrap HP 5mL
Lysis buffer	50mM Tris, 500mM NaCl , 25mM Imidazole, 20mM β -mercaptoethonal , 0.2mM PMSF, pH8.0
Ni Buffer	**A**: 50mM Tris, 500mM NaCl , 25mM Imidazole, 20mM β -mercaptoethonal , pH8.0 **B**: 50mM Tris, 1M NaCl , 25mM Imidazole, 20mM β -mercaptoethonal , pH8.0 **C**:50mM Tris, 500mM NaCl , 25mM Imidazole, 20mM β -mercaptoethonal, pH8.0
Elution	Target proteins were eluted with a line gradient of 4.5-75% buffer C in 10CV.





Pool 19-25 (~40mg)

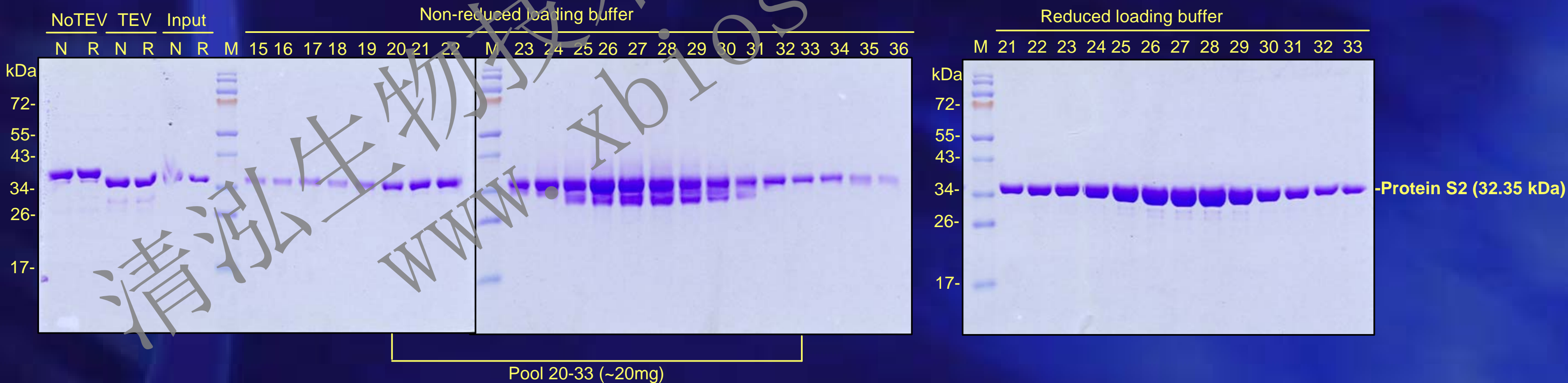
TEV cleavage
50ug/mg protein
Overnight at 4℃

NI: non-induced
I: induced
P: cell pellets
S: cell supernatant

清泓生物技术（上海）有限公司 www.xbioservice.com

Purification step two: TEV&Q-Column

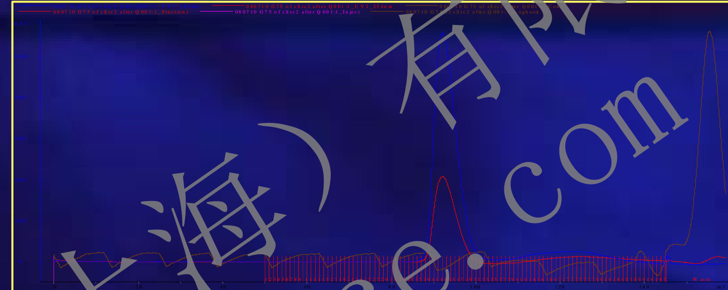
Sample	Protein S2 after TEV cleavage
Column	HiTrap Q FF 5mL
Q Buffer	**A**: 50mM Tris, 20mM NaCl ,5mM DTT , pH8.0 **B**: 50mM Tris, 1M NaCl , 5mM DTT , pH8.0
Elution	Target proteins were eluted with a line gradient of 0-20% buffer C in 10CV.

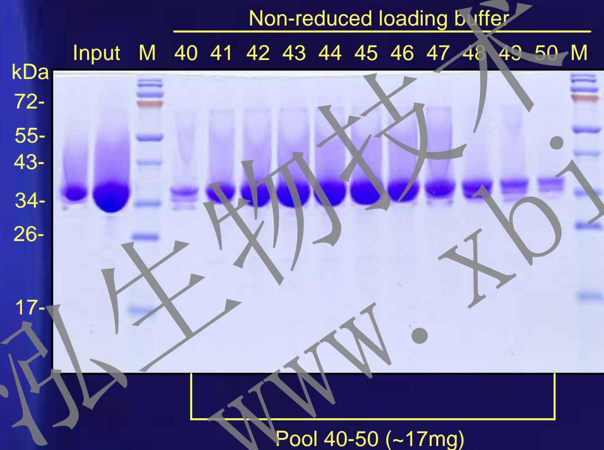


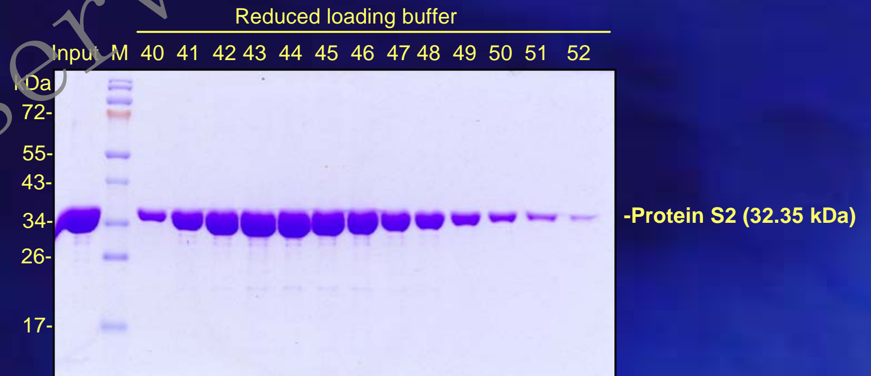
清泓生物技术（上海）有限公司 www.xbioservice.com

Purification step three: Sizing Column

Sample	Protein S2 Q-Column pool 20-33
Column	Superdex 75
Buffer	50mM Tris, 100mM NaCl ,5mM DTT , pH8.0





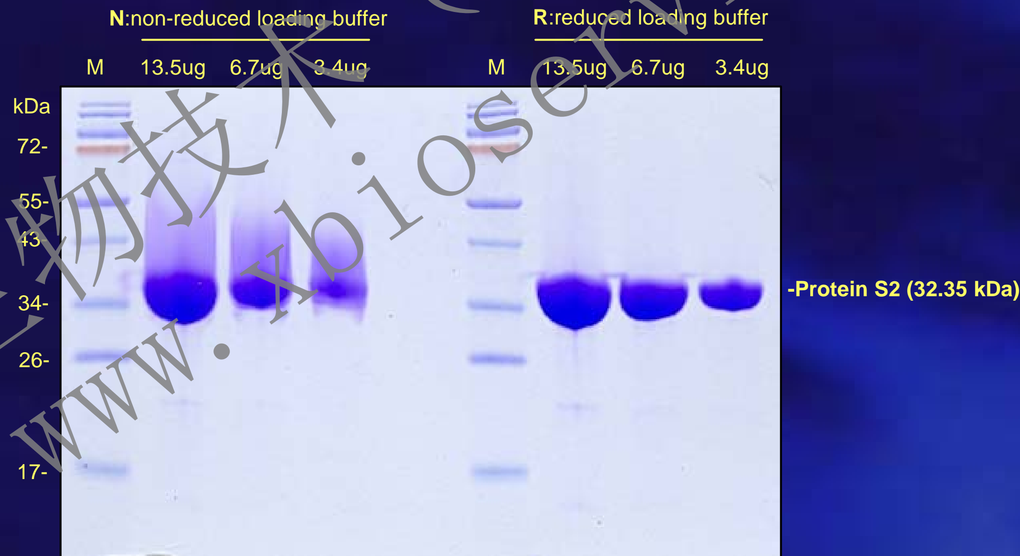


清泓生物技术（上海）有限公司 www.xbioservice.com

Purification step four: Concentration

	Concentration .(mg/ml)	Volume (ml)	Total（mg）	method
Protein S2	9	0.6	5.40	Bradford*
Buffer	50mM Tris, 100mM NaCl, 5mM DTT, pH8.0			

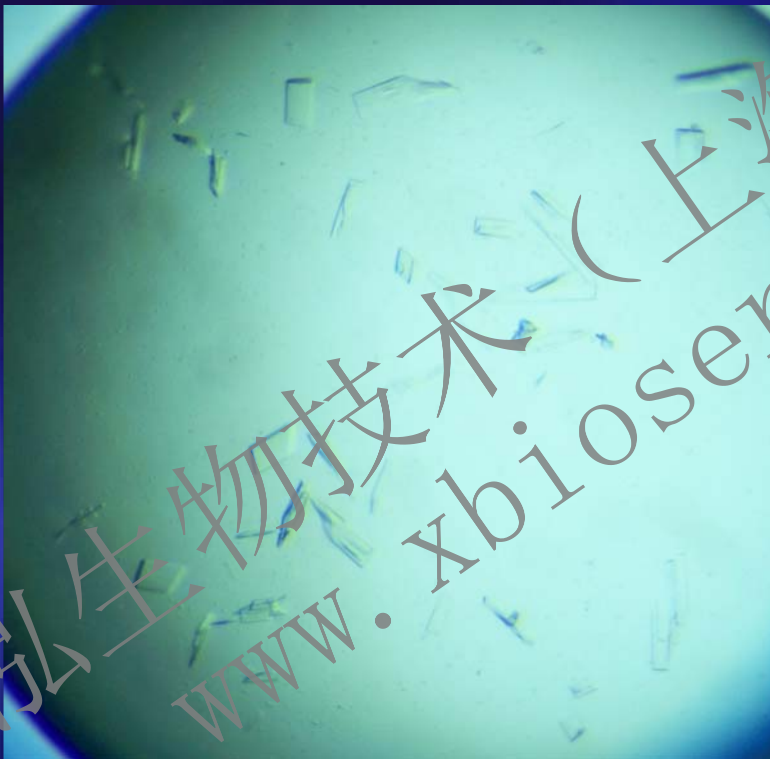
Note, BSA is always used as the reference protein in Bradford method to measure the protein concentration at SG.



清泓生物技术（上海）有限公司 www.xbioservice.com

Better crystals of native Protein S grew in the 2nd round of screening

Crystals of native Protein S2, 60x



Native Protein S2

Temperature: 18℃

Protein concentration: 3.7 mg/ml

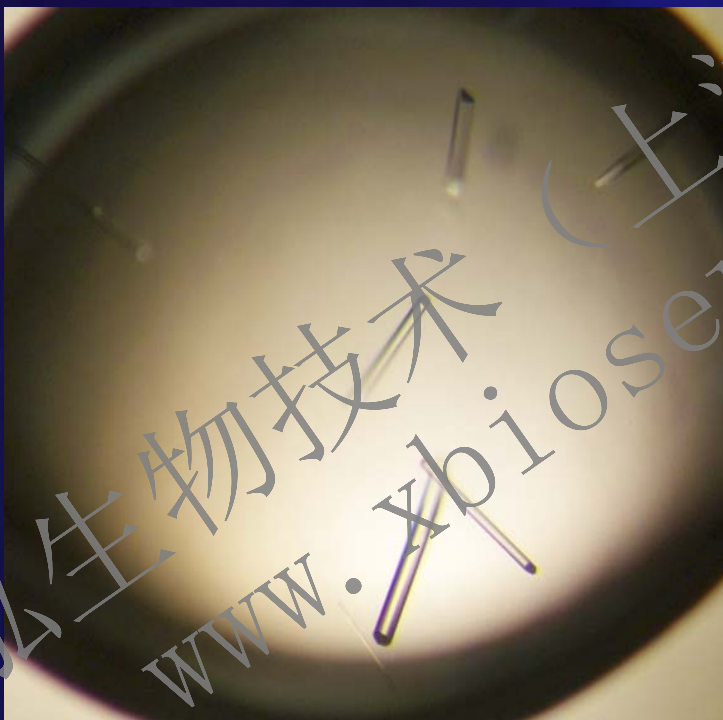
Reservoir solution: IHT G11,
0.2 M Magnesium Chloride hexahydrate
0.1 M Bis-Tris pH 6.5
25% w/v PEG 3350

Diffraction data was collected with resolution at 2.63 Å.

清泓生物技术（上海）有限公司
www.xbioservice.com

Better crystals of Protein S co-crystallized with compound 43 was obtained when using additive to optimize the crystallization

Crystals of Protein S2 & 43, 60x



Protein S2 & 43

Temperature: 18℃

Reservoir solution: IHT G11,
0.2 M Magnesium Chloride hexahydrate
0.1 M Bis-Tris pH 6.5
25% w/v PEG 3350

Additive: C6
30% w/v 6-Aminohexanoic acid
From Additive Screen ™ kit
(Hampton Research Corp, HR2-428)

Mixture: 1 μl of protein, 0.8 μl of reservoir solution and 0.2 μl of additive were used.

Diffraction data was collected with resolution at 1.8 Å.

清泓生物技术（上海）有限公司
www.xbioservice.com

Strong and clear electric density map may be found around the compound binding area

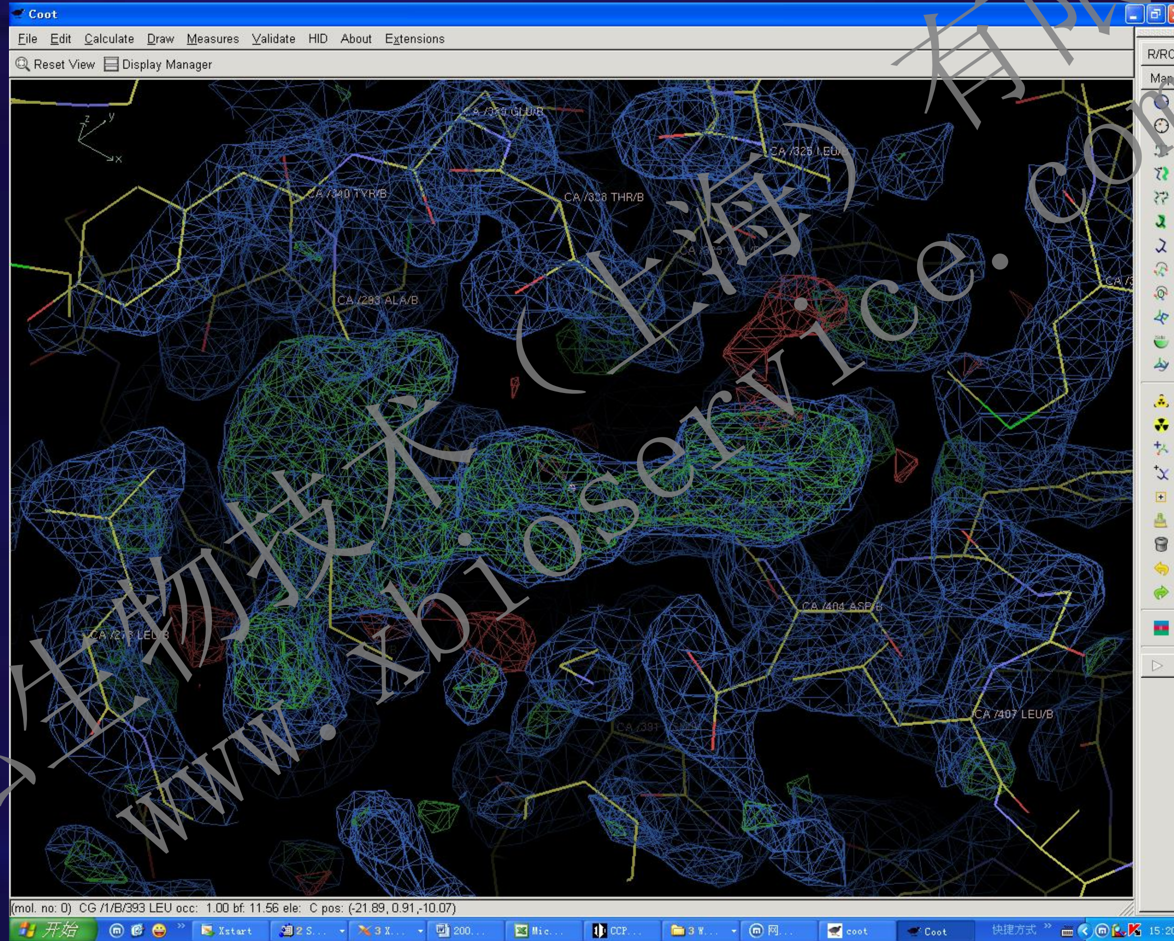


Fig 1: Strong and clear electric density map around the compound binding area (1.00 sigma 2Fo-Fc map in blue and 3.00 sigma Fo-Fc map in green)

清泓生物技术（上海）有限公司
www.xbioservice.com

Summary for case study

- Protein S protein was purified with good purity and yield.
- Succeed in crystallization of native Protein S kinase, high quality diffraction data was collected.
- Succeed in crystallization of Protein S kinase complexed with compound 43, excellent quality diffraction data was collected.

清泓生物技术（上海）有限公司
www.xbioservice.com