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BIO BASIC INC.

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# Focus on Biological Purification Products


*QF 24 TV6*
*CV2 2021*


***For Research Use Only***

# Focus on Biological Purification Products

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# Affinity Chromatography for Fusion Protein

## 1. Histidine tag (6×his tag) fusion protein

Immobilized metal ion affinity chromatography (IMAC) is a widely used separation method for purifying a broad range of protein and peptides. It is based on specific interaction between certain amino acid chains (such as histidine, tryptophan, L-cysteine, etc.) exposed on the surface of protein and transition metal ions. The IMAC medium is widely used to separate the proteins with histidine chains. The key features of the IMAC include high binding capacity, high stable, mild separation conditions and fast reliable scale-up of his-tagged protein purification. In recent years, IMAC has been increasingly used for the purification of histidine-tagged proteins expressed in series of expression systems, such as E.coli, yeast, insect cells and mammalian cells.

Ni smart beads and Ni smart beads 6FF are new immobilized metal ion affinity chromatography (IMAC) medium pre-charged with nickel ions. They are designed mainly for capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. The strong nickel ion binding also provides very high resistance to EDTA and reducing agents like DTT. They enable direct loading of large sample volumes without having to remove agents that cause stripping of nickel ions from conventional IMAC medium. Ni Smart Beads are stable in all buffers commonly used IMAC.

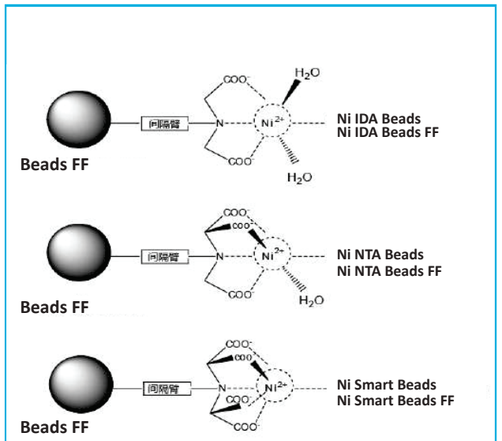



**Fig. 2-1 Schematic diagram of ligand of IMAC medium**

See table below for reagent tolerance.

| Reagent | Tested time |
|---|---|
| 0.01M HCl, 0.01M NaOH | One week |
| 10mM EDTA, 1M NaOH, 5mM DTT, 5mMTCEP, 20mMMercaptoethanol 6M Guanidine hydrochloride | 24 hours |
| 500 mM imidazole, 100mM EDTA | 2 hours |
| 30% Isopropanol | 20 min |

**Table 2-1 Chemical stability of Ni Smart Beads and Ni Smart Beads 6FF**



### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| Ni NTA Beads 6FF | Ni NTA Beads 6FF is compatible with native or denaturing conditions and can be used in FPLC. It is easy to pack and use. Its high flow properties make excellent for scaling-up. | > 40mg 6×his-tagged protein | MSA005010 | 10ml | $170.18 | $130.91 |
| | | | MSA005025 | 25ml | $378.18 | $290.91 |
| | | | MSA005100 | 100ml | $1252.73 | $963.64 |
| | | | MSA005500 | 500ml | $4697.73 | $3613.64 |

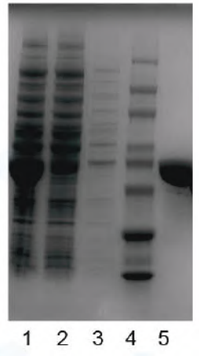
### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| Ni Smart Beads 6FF | Ni smart beads and 6FF are new immobilized metal ion affinity chromatography (IMAC) medium precharged with nickel ions. The strong nickel ion binding make sure that the medium can purify directly without buffer exchange and concentration procedures. | > 10mg 6×his-tagged protein | MSA036010 | 10ml | $246.76 | $189.82 |
| | | | MSA036050 | 50ml | $992.73 | $763.64 |
| | | | MSA036100 | 100ml | $1829.45 | $1407.27 |
| | | | MSA036500 | 500ml | $6860.45 | $5277.27 |

## 2. GST Fusion Protein

Glutathione Beads and Glutathione Beads 4FF are the affinity chromatography for purification of glutathione S-transferase (GST), Glutathione-dependent protein and GST-tagged protein expressed in various expression systems. The reduced glutathione ligand is coupled to the agarose beads via a 12-atom linker. The coupling is optimized to give high binding capacity for GST-tagged proteins.

Glutathione Beads 4FF can be used in FPLC. It is easy to pack and use. Its high flow properties make it excellent for scaling-up.




**Name:** Glutathione Beads
**Sample:** GST tag fusion protein
**BindingBuffer:** 140mM Nacl, 2.7mM Kcl, 10mM Na2HPO4, 1.8mM KH2PO4, pH7.4
**Elution buffer:** 50mM Tris-HCl, 10mM reductive Glutathione, pH8.0
**Lane1**: GST tag protein lysate
**Lane2:** Sample flow
**Lane3:** Washing
**Lane4:** Protein Marker
**Lane5:** Elution

**Fig. 2-4 SDS-PAGE analysis of purification of GST fusion protein**

### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| Glutathione Beads 4FF | Glutathione Beads have high binding capacity of GST-tagged protein. | >20mgGST-tagged protein (40Da) | MSA010010 | 10ml | $260.00 | $200.00 |
| | | | MSA010050 | 50ml | $992.73 | $763.64 |
| | | | MSA010100 | 100ml | $1796.36 | $1381.82 |
| | | | MSA010500 | 500ml | $6736.36 | $5181.82 |

## 3. MBP Fusion Protein

The molecular weight of maltose binding protein (MBP) is about 42000Da. MBP labeling can promote the fusion protein with correct folding, increase the expression level and solubility of the target protein. MBP is particularly useful for recombinant proteins accumulated in an insoluble form (inclusion bodies).

Dextrin Beads 6FF is an affinity chromatography medium for purification of MBP-tagged proteins. MBP fusion proteins can be purified in one step. The binding fusion proteins can be gently eluted with 10 mm maltose to protect the activity of target proteins. MBP can be removed from the fusion protein by site-specific protease.


**Medium: Dextrin Beads 6FF**
**Sample:** MBP fusion protein in E.coli lysate
**BindingBuffer:** 20mM Tris-HCl, 200mM Nacl, 1mM EDTA, pH7.4
**Elution buffer**: 20mM Tris-HCl, 1mM EDTA, 10mM Maltose, pH7.4
**Lane1:** Sample
**Lane2:** Flow through
**Lane3:** Elution 1
**Lane4:** Elution 2
**Lane5:** CIP

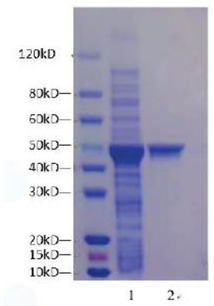
**Fig. 2-5 SDS-PAGE analysis of purification of MBP fusion protein**

### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| Dextrin Beads 6FF | The medium is compatible with commonly used buffer and can be regenerated with 0.5M NaOH Its high flow proper-ties make it excellent for scaling-up detergents and other additives. | > 10mg MBP fusion protein | MSA026010 | 10ml | $165.45 | $127.27 |
| | | | MSA026025 | 25ml | $354.55 | $272.73 |
| | | | MSA026100 | 100ml | $1063.64 | $818.18 |

## 4. Biotin Fusion Protein

Streptavidin Beads 6FF is an affinity chromatography due to the interaction between biotin and streptavidin. The interaction between streptavidin and biotin is very strong and requires denaturating conditions for elution, which may destroy both the ligand and the sample. The interaction between 2-iminobiotin and streptavidin is weak, it can be eluted at pH4.0, which preserve the biomolecules activity.The crosslinking of the base matrix has been optimized to give the matrix good flow properties and high physical and chemical stability, both of which are key factors for cost-effective large-scale use.




**Medium:** Streptavidin Beads 6FF
**Sample:** 2-iminobiotinylated protein
**Binding/Washing:** 50mM boric acid, 0.5M NaCl, pH10.0
**Elution buffer:** 50mM sodium acetate, 0.5M NaCl, pH4.0
**Lane1:** Sample
**Lane2:** Elution fraction
**Fig. 2-6 SDS-PAGE analysis of purification of iminobiotinylated protein**

### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| Streptavidin Beads 6FF | Streptavidin Beads 6FF can be used for immobilization or detection of biotinylated nucleotides, peptides, proteins and cells ,etc. | 6mg Biotinylated serum protein | MSA021005 | 5ml | $212.73 | $163.64 |
| | | | MSA021010 | 10ml | $340.36 | $261.82 |
| | | | MSA021100 | 100ml | $2893.09 | $2225.45 |



# Affinity Chromatography for Antibody

According to the different application, Bio Basic has three categories products for purification of general antibody, monoclonal antibody and polyclonal antibody.

## General Antibody

The ligand of products for the purification of general antibody are mainly Protein A and Protein G. They can also be used for the purification of polyclonal and monoclonal antibody.

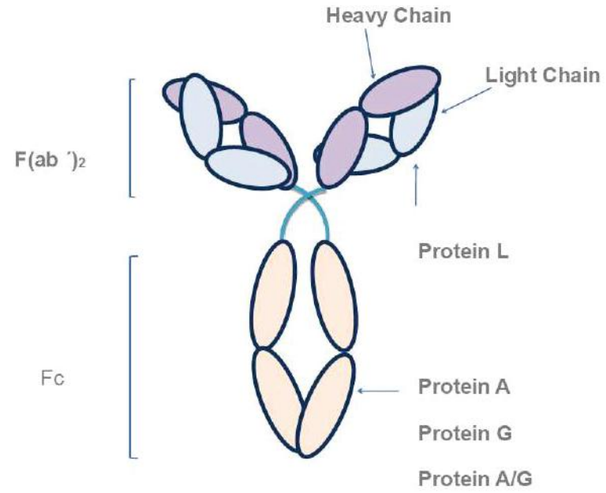



**Fig. 3-1 the site of the antibody bind to the protein**

The binding capacity of protein A and protein G to immunoglobulins from different species and subclasses are different. The specific binding properties are shown in the table below.

| Species and Genera | Subtype | Protein A Binding Force | Protein G Binding Force |
|---|---|---|---|
| Human | IgA | varible | — |
| | IgD | — | — |
| | IgE | — | — |
| | IgG1 | ++++ | ++++ |
| | IgG2 | ++++ | ++++ |
| | IgG3 | — | ++++ |
| | IgG4 | ++++ | ++++ |
| | IgM | varible | — |
| Avian egg yolk | IgY | — | — |

| Species and Genera | Subtype | Protein A Binding Force | Protein G Binding Force |
|---|---|---|---|
| Cow | | ++ | ++++ |
| Dog | | ++ | + |
| Goat | | — | ++ |
| Guinea pig | IgG1 | ++++ | ++ |
| | IgG2 | ++++ | ++ |
| Hamster | | + | ++ |
| Horse | | ++ | ++++ |
| Koala | | — | + |
| Liama | | — | + |
| Monkey(rhesus) | | ++++ | ++++ |
| Guinea pig | IgG1 | ++++ | ++ |
| | IgG2 | ++++ | ++ |
| Hamster | | + | ++ |
| Horse | | ++ | ++++ |
| Koala | | — | + |
| Liama | | — | + |
| Monkey(rhesus) | | ++++ | ++++ |
| Mouse | IgG1 | + | ++++ |
| | IgG2a | ++++ | ++++ |
| | IgG2b | +++ | +++ |
| | IgG3 | ++ | +++ |
| | IgM | varible | — |
| Pig | | +++ | +++ |
| Rabbit | no distinction | ++++ | +++ |
| Rat | IgG1 | — | + |
| | IgG2a | — | ++++ |
| | IgG2b | — | ++ |
| | IgG3 | + | ++ |
| Sheep | | +/- | ++ |

++++=Strong binding; ++=medium binding; -=weak or no binding

## 1. Protein A Related Products

rProtein A Beads and rProtein A Beads 4FF are one of the universal affinity chromatography media for the separation and purification of antibodies. Protein A is a cell wall protein isolated from Staphylococcus aureus. It binds mammalian IgG mainly through Fe fragment, but not human IgM, IgD and IgA. Natural protein A has five IgG binding regions and some unknown functional regions. Recombinant protein A has only five IgG binding regions, which reduces the nonspecific adsorption.

The recombinant protein A ligand is coupled to highly cross-linked 4% agarose beads. The coupling is optimized to give high binding capacity for immunoglobulins. The static binding capacity of rProtein A Beads 4FF is greater than 40 mg human IgG/ml settled beads. The product can be used for one-step purification of monoclonal antibody and polyclonal antibody, and the purity can reach 95%.

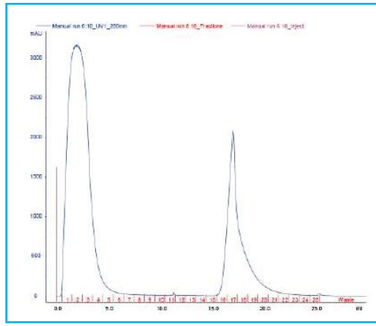


### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| rProtein A Beads 4FF | rProtein A Beads 4FF can be used in FPLC. It is easy to pack and use. Its high flow properties make it excellent for scaling-up. | >40mg human IgG | MSA015005 | 5ml | $260.00 | $200.00 |
| | | | MSA015025 | 25ml | $1087.27 | $836.36 |
| | | | MSA015200 | 200ml | $3309.09 | $2545.45 |

## 2. Protein G Related Products

rProtein G Beads and rProtein G beads 4FF are used to separate and purify IgG. Protein G is used as affinity ligand. Protein G is a cell wall protein isolated from G streptococci. It can bind to mammalian IgG through its Fe fragment. The recombinant protein G contained high affinity binding sites, which reduced the nonspecific adsorption. Protein G and protein A have different binding properties to IgG. Compared with protein A, protein G has stronger binding power to polyclonal antibodies such as cattle, sheep and horses.



**Column:** AbCap G4FF 1ml
**Sample:** Mouse ascities 2ml
**Binding/washing buffer:** 20mM phosphate, 0.01M NaCl, PH7.0
**Elution Buffer:** 0.1M Glycine, PH 3.0
**Lane1:** Sample
**Lane2:** Flow through
**Lane3:** Elution fractions

**Fig. 3-2 Purification of antibody by AbCap G 4FF**

It can also bind rat IgG, human IgG3 and mouse IgG1 which can not bind well with protein A.

### Product Information

| Product name | Applications | Characteristics (/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|
| rProtein G Beads 4FF | rProtein G Beads 4FF can be used in FPLC. It is easy to pack and use. Its high flow properties make it excellent for scaling-up. | >30mg human IgG | MSA020005 | 5ml | $260.00 | $200.00 |
| | | | MSA020025 | 25ml | $1087.27 | $836.36 |
| | | | MSA020100 | 100ml | $3309.09 | $2545.45 |
| | | | MSA020200 | 200ml | $4609.09 | $3545.45 |
| | | | MSA020500 | 500ml | $9762.55 | $7532.73 |

## 3. Protein L Related Products

rProtein L Beads and rProtein L beads 4FF are universal affinity chromatography media for easy, one-step purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. Unlike Protein A and Protein G, which bind primarily through Fe regions of immunoglobulins, Protein L binds the variable region of antibodies kappa light chain and binds a wider range of Ig classes and subclasses. Protein L is therefore particular suitable for capture of a wide range of different-sized antibody fragments such as Fab fragments, single-chain variable fragments (Scfv) and domain antibodies(dAbs).

### Product Information

| Product name | Applications | Characteristics (/ml medium) | Recommended Flow Rate(/ml medium) | Cat. No. | Size | List Price (CAD) | List Price (USD) |
|---|---|---|---|---|---|---|---|
| rProtein L Beads 4FF | rProtein L is obtained by gene recombination technology rProtein L Beads 4FF bind the variable region of antibodies kappa light chain and the affinity did not affect the antigen binding with antibody. So it binds a wider range of Ig classes and subclasses. | >15mg IgG | 300-500 | MSA033005 | 5ml | $626.36 | $481.82 |
| | | | | MSA033025 | 25ml | $2836.36 | $2181.82 |
| | | | | MSA033300 | 200ml | $10163.64 | $7818.18 |