Compatibility of reagents with Ni-NTA

Reagent	Effect	Comments
Buffer reagents		
Tris, HEPES, MOPS	Buffers with secondary or tertiary amines may reduce nickel ions.	Up to 100 mM can be used, however sodium phosphate or phosphate-citrate buffer is recommended.
Chelating reagents		
EDTA, EGTA	Strips nickel ions from resin.	Up to 1 mM has been used successfully in some cases, but care must be taken.
Sulfhydril reagents		
β-mercaptoethanol	Prevents disulfide cross-linkages. Can reduce nickel ions at higher concentration.	Up to 20 mM can be used. Do not store resin under reducing conditions.
DTT, DTE	At high concentrations (>1 mM) resin may turn reversibly brown due to nickel reduction. Up to 10 mM has been tested and shown not to compromise purification or increase nickel leaching.	Up to 10 mM DTT has been used successfully. Do not store resin under reducing conditions.
TCEP	Prevents disulfide cross-linkages.	Up to 1 mM tested successfully. Do not store resin under reducing conditions.

Reagent	Effect	Comments
Nonionic detergents		
n-Hexadecyl-β-D-maltoside	Removes background proteins and nucleic acids, resolubilizes membrane proteins from membrane compartments.	0.0003%*
n-Tetradecyl-β-D-maltopyranoside		0.005%*
n-Tridecyl-β-D-maltopyranoside		0.016%*
Brij 35		0.1%*
Digitonin		0.6%*
Cymal 6, n-Nonyl-β-D-glucopyranoside, n-Decyl-β-D-maltopyranoside, n-Dodecyl-β-D-maltoside, C12-E9		1%*
n-Octyl-β-D-glucopyranoside		1.5%*
Triton®, Tween®, NP-40		Up to 2% can be used.
Zwitterionic detergents		
Fos-Choline 16		0.05%*
Dodecyldimethylphosphineoxide		0.15%*
Cationic detergents		Up to 1% can be used.
CHAPS		Up to 1% can be used.

* Highest concentration tested at QIAGEN. Maximum concentration compatible with Ni-NTA may be higher.

Reagent	Effect	Comments
Anionic detergents (SDS, sarkosyl)		Not recommended, but up to 0.3% has been used successfully in some cases.
Triton X-114	Removes endotoxins	Up to 2% can be used.
Denaturants		
GuHCl	Solubilizes proteins	Up to 6 M.
Urea		Up to 8 M.
Amino acids		
Glycine		Not recommended.
Glutamine		Not recommended.
Arginine		Not recommended.
Histidine	Binds to Ni-NTA and competes with histidine residues in the His tag. Elution with histidine can help to reduce aggregation of eluted protein.	Can be used at low concentrations (1–2 mM) to inhibit non specific binding and, at higher concentrations (>20 mM), to elute the His-tagged protein from the Ni-NTA matrix.
Other additives		
NaCl	Prevents ionic interactions.	Up to 2 M can be used, at least 300 mM should be used.
$MgCl_2$		Up to 4 M.
$CaCl_2$		Up to 5 mM.
Glycerol	Prevents hydrophobic interaction between proteins, stabilizes proteins.	Up to 50%.

Reagent	Effect	Comments
Ethanol	Prevents hydrophobic interactions between proteins.	Up to 20%.
BugBuster® Protein Extraction Reagent		Use as recommended.
Imidazole	Binds to Ni-NTA and competes with histidine residues in the His tag.	Can be used at low concentrations (20 mM) to inhibit non specific binding and, at higher concentrations (>100 mM), to elute the His-tagged protein from the Ni-NTA matrix.
Sodium bicarbonate		Not recommended.
Hemoglobin		Not recommended.
Ammonium		Not recommended.
Citrate	Buffer	Up to 60 mM has been used successfully.

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