Ni Sepharose™ excel (25, 100 and 500 ml) HisTrap™ excel (1 and 5 ml)

Ni Sepharose excel is an immobilized metal ion affinity chromatography (IMAC) medium (resin) precharged with nickel ions. The medium is designed mainly for capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. The very strong binding of nickel ions to Ni Sepharose excel enables direct loading of large sample volumes without having to remove agents that cause stripping of nickel ions from conventional IMAC media. The strong nickel ion binding also provides very high resistance to EDTA and reducing agents like DTT. Ni Sepharose excel is also suitable for purification of histidine-tagged proteins from other samples, including *E. coli* lysates.

The medium is available in the following formats:

- 25, 100 and 500 ml medium packs for packing in chromatography columns, such as Tricorn™, XK or HiScale™ columns.
- HisTrap excel, 1 ml and 5 ml columns prepacked with Ni Sepharose excel.

The special type of filters in the top and bottom of HisTrap excel columns makes it possible to directly load cell-free, unclarified samples on the columns without causing back pressure problems.

The HisTrap excel columns can be operated with a peristaltic pump or liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

1 Product description

Background

Traditionally, IMAC purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants is often problematic due to incompatibility with the cell culture media, leading to stripping of the immobilized metal ions during sample loading. This results in low or no binding of the target protein.

The purification is further complicated by the fact that the target protein concentration often is low. This requires large sample volumes to be used, which in turn may lead to increased metal ion stripping.

To overcome these problems, considerable sample pretreatment has been required, such as buffer exchange by ultrafiltration in combination with concentration procedures. Such pretreatment is time consuming and potentially harmful to sensitive proteins.

General description

Ni Sepharose excel and HisTrap excel (columns prepacked with Ni Sepharose excel) are products for IMAC protein purification. Ni Sepharose excel consists of 90 µm highly cross-linked agarose beads, to which a chelating ligand has been coupled. The ligand is precharged with exceptionally strongly bound nickel ions. The nickel ions remain bound even after 24 hours incubation in 10 mM EDTA. No nickel ion re-charging is required after use.

Samples usually causing stripping of metal ions can be loaded onto the medium without extensive and time-consuming pretreatment procedures. Examples of samples that often cause stripping problems are cell culture supernatants containing secreted histidine-tagged proteins from eukaryotic cells, such as insect cells or CHO cells. Ni Sepharose excel and HisTrap excel are also suitable for purification of histidine-tagged proteins from other samples causing extensive metal ion stripping from conventional IMAC media.

For samples without extensive stripping effects, it is recommended to use Ni Sepharose 6 Fast Flow or Ni Sepharose High Performance, which normally show higher affinity for histidine-tagged proteins.

Ni Sepharose excel

Characteristics

The flow properties of Ni Sepharose excel make it excellent for purifications in all scales and allow loading of large sample volumes. Due to low target protein concentrations, several liters often need to be processed when purifying secreted proteins.

Tricorn, XK and HiScale columns are recommended for packing of the medium.

The key characteristics of Ni Sepharose excel are listed in the table below.

Ni Sepharose excel characteristics

Matrix	Highly cross-linked spherical agarose, 6%
Average particle size	90 µm
Metal ion capacity	54 to 70 µmol Ni^{2+}/ml medium
Dynamic binding capacity [1, 2]	At least 10 mg $(histidine)_6$-tagged protein/ml medium
Recommended flow velocity [3, 4]	150 to 600 cm/h
Maximum flow velocity [3]	600 cm/h
pH stability [5]	
Working range	2 to 12
Cleaning-in-place	2 to 14
Compatibility during use	Stable in all buffers commonly used in IMAC
Chemical stability [6]	
- 0.01 M HCl and 0.01 M NaOH	Tested for one week
- 10 mM EDTA, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol, 1 M NaOH, and 6 M guanidine-HCl	Tested for 24 hours
- 500 mM imidazole and 100 mM EDTA	Tested for 2 hours
- 30% 2-propanol	Tested for 20 minutes
Storage	20% ethanol at 4°C to 30°C

1 Binding capacity is sample and protein dependent. The binding capacity for most proteins is considerably higher than 10 mg/ml.

[2] Dynamic binding capacity was tested with 0.5 mg/ml pure $(histidine)_6$-tagged protein (M_r 43 000) or $(histidine)_6$-tagged protein (M_r 28 000) spiked in EX-CELL™ 420 Insect serum-free medium (capacity at 10% breakthrough). Column volume was 1 ml and flow rate 1 ml/min.

[3] H_2O at room temperature. For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[4] Optimal flow velocity during binding is sample-dependent. During column wash and elution a flow velocity of 150 cm/h is recommended. Do not exceed 150 cm/h during cleaning-in-place (CIP).

[5] Working range: pH interval where the medium can be handled without significant change in function.
Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

[6] Chemical stability was tested by incubating the medium in the listed solutions at room temperature, and thereafter measuring either the nickel leakage or the protein binding capacity.

HisTrap excel

Characteristics

HisTrap excel, 1 ml and 5 ml, are ready-to-use IMAC columns prepacked with Ni Sepharose excel. The design of the columns in combination with the specific properties of the medium enables fast and convenient purifications.

The special type of filters in the top and bottom of the columns makes it possible to directly load cell-free, unclarified samples on the columns without causing back pressure problems. This is time-saving, which helps to prevent degradation and thus loss of sensitive target proteins.

HisTrap excel columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet. HisTrap excel columns cannot be opened or refilled.

The columns can be operated with either a peristaltic pump or chromatography systems such as ÄKTA systems. For easy scaling-up, two HisTrap excel columns can simply be connected in series.

The key characteristics of the HisTrap excel columns are listed in the following table. For characteristics of the Ni Sepharose excel medium used in the columns, see table on page 4.

HisTrap excel characteristics

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Recommended flow rate [1, 2]	1 to 4 ml/min	5 to 20 ml/min
Maximum flow rate [1]	4 ml/min	20 ml/min
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

[1] H_2O at room temperature. Maximum flow rate will be lower when using buffers or samples with high viscosity or performing purification at low temperature.

[2] Optimal flow rate during binding is sample-dependent. During column wash and elution, a flow rate of 1 ml/min and 5 ml/min is recommended for 1 ml and 5 ml columns, respectively. During CIP, do not exceed a flow rate of 1 ml/min and 5 ml/min for 1 ml and 5 ml columns, respectively.

Note: *The maximum pressure the packed bed can withstand depends on the chromatography medium characteristics and sample/liquid viscosity. The value measured on the chromatography system used also depends on the tubing used to connect the column.*

Supplied Connector kit with HisTrap excel columns

Connectors supplied	**Usage**	**No. supplied**
Union 1/16" male/luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

2 Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.45 µm filter before use. Use high purity imidazole, which gives essentially no absorbance at 280 nm.

Recommended buffers

Equilibration buffer
20 mM sodium phosphate, 0.5 M NaCl, pH 7.4

Wash buffer
20 mM sodium phosphate, 0.5 M NaCl, 0 to 30 mM imidazole, pH 7.4

Elution buffer
20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Note: *It is not recommended to include imidazole in sample and equilibration buffers.*

Note: *Optimal imidazole concentration during wash is sample-dependent. See below for further information.*

Buffer optimization

Imidazole concentration in wash buffers

To minimize host cell proteins in the eluate, it is recommended to include imidazole at low concentrations in the wash buffer. However, for some target proteins, even a small increase of the imidazole concentration in the wash buffer may lead to partial elution.

The imidazole concentration must therefore be optimized to ensure the best balance of high purity (low binding of host cell proteins) and high yield (strong binding of histidine-tagged target protein). For high purity, a higher imidazole concentration is required in the wash buffer, and insect cell culture supernatants generally require higher imidazole concentration than CHO cell culture supernatants.

The concentration of imidazole that will give optimal purification results is sample-dependent, and is usually lower for Ni Sepharose excel than for other Ni Sepharose media used for IMAC purification. Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 10 to 20 mM in the wash buffer is a good starting range for many proteins.

Eliminating ion-exchange effects

Addition of salt, for example 0.5 to 1.0 M NaCl in buffers eliminates ion-exchange effects.

Elution

In general, imidazole is used for elution of histidine-tagged proteins. Alternatively, the proteins may be eluted by other methods or combinations of methods, for example by lowering pH within the range 2.5 to 5.0.

With Ni Sepharose excel no recharging with nickel ions between each run is required when elution is performed using either low pH or imidazole. This is in contrast to many other IMAC media, which often have to be recharged with nickel ions after elution using low pH.

Note: *If the proteins are sensitive to low pH, it is recommended to collect the pH-eluted fractions in tubes containing a suitable volume of 1 M Tris-HCl, pH 9.0 to restore a neutral pH.*

3 Sample preparation

Before sample loading, whole cells must be removed by, for example, centrifugation, otherwise clogging of the column may occur. When using HisTrap excel columns, no further clarification is needed. When using Ni Sepharose excel packed in other columns, it is recommended to also filter the sample through a 0.45 µm filter to remove cell debris and/or other particulate material.

For optimal binding, it is **not** recommended to include imidazole in sample and equilibration buffer. It is recommended to perform binding at neutral pH. However, successful purification has routinely been observed with binding performed at a pH as low as 6.0. Due to the precipitation risk, avoid using strong bases or acids for pH-adjustments.

4 Ni Sepharose excel protocols

Column packing

Ni Sepharose excel is supplied pre-swollen in 20% ethanol.

Suitable columns for packing are the Tricorn, XK and HiScale columns, see Section *Ordering information*.

Preparation

Step	Action
1	Allow the medium slurry to sediment for 3 hours.
2	Add enough 20% ethanol to achieve a 1:1 ratio of settled medium and overlayed 20% ethanol.
3	Assemble the column (and packing reservoir if necessary).[1]
4	Remove air from the end-piece and adapter by flushing with 20% ethanol. *Make sure no air has been trapped under the column bed support*.
5	Close the column outlet leaving the bed support covered with 20% ethanol.

[1] If the purification is to be performed at a high flow rate, HiScale columns are recommended.

Packing

Step	Action
1	Resuspend the medium and pour the slurry into the column in a single continuous motion. *Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.*
2	Allow the medium slurry to sediment for at least 3 hours.
3	If using a packing reservoir, disconnect the reservoir and fill the remainder of the column with 20% ethanol.
4	Mount the adapter and connect the column to a pump. *Avoid trapping air bubbles under the adapter or in the inlet tubing.*
5	Immediately open the bottom outlet of the column and set the pump to run at the desired flow rate. [1]
6	Maintain packing flow rate for at least 3 column volumes (CV) after a constant bed height is reached.
7	Stop the pump and close the column outlet.
8	With the adapter inlet disconnected, quickly push the adapter down into the column until it reaches the bed surface and then a further 3 to 4 mm into the medium bed. Lock the adapter at this level.
9	Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

[1] Should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. If this cannot be obtained, use the maximum flow rate your pump can deliver. This should also give a well-packed bed.

Purification protocol for Ni Sepharose excel

Note: *For purification protocol for HisTrap excel, see next Section.*

Step	Action
1	If the column contains 20% ethanol, wash it with 5 column volumes (CV) distilled water. *Recommended flow velocity: 50 to 100 cm/h.*
2	Equilibrate the column with at least 5 CV equilibration buffer. *Recommended flow velocity: 150 to 600 cm/h.*
3	Load the sample. *Recommended flow velocity: 150 to 600 cm/h.*
4	Wash with 20 CV wash buffer. *Recommended flow velocity: 150 cm/h.*
5	Elute with elution buffer using a one-step procedure. 5 CV elution buffer is usually sufficient. Alternatively, a linear elution gradient (10 to 20 CV) may give higher purity, at the expense of lower target protein concentration in eluted fractions. *Recommended flow velocity: 150 cm/h.*

Note: *A lower flow rate during sample loading might result in higher yield of the target protein.*

Note: *Purification at low temperatures increases the sample and buffer viscosity, leading to increased back pressure. If necessary, decrease flow rate.*

Note: *If imidazole needs to be removed from the protein after the purification, prepacked desalting columns such as HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 Desalting columns can be used, see Ordering information.*

5 HisTrap excel protocol

Purification protocol

Step	Action
1	Fill the pump tubing with distilled water. Remove the stopper and connect the column to the chromatography system or the laboratory pump "drop-to-drop" to avoid introducing air into the column. Make sure that the connector is tight to prevent leakage.
2	Remove the snap-off end at the column outlet.
3	Wash out the ethanol with 5 column volumes (CV) distilled water. *Recommended flow rate: 1 ml/min.*
4	Equilibrate the column with at least 5 CV equilibration buffer. *Recommended flow rates: 1 to 4 ml/min and 5 to 20 ml/min for the 1 ml and 5 ml columns, respectively.*
5	Load the sample. *Recommended flow rates: 1 to 4 ml/min and 5 to 20 ml/min for the 1 ml and 5 ml columns, respectively.*
6	Wash with 20 CV wash buffer. *Recommended flow rates: 1 ml/min and 5 ml/min for the 1 ml and 5 ml columns, respectively.*
7	Elute with elution buffer using a one-step procedure. 5 CV elution buffer is usually sufficient. Alternatively, a linear elution gradient (10 to 20 CV) may give higher purity, at the expense of lower target protein concentration. *Recommended flow rates: 1 ml/min and 5 ml/min for the 1 ml and 5 ml columns, respectively.*

Note: *A lower flow rate during sample loading might result in higher yield of the target protein.*

Note: *Purification at low temperatures increases the sample and buffer viscosity, leading to increased back pressure. If necessary, decrease flow rate.*

Note: *If imidazole needs to be removed from the protein after the purification, prepacked desalting columns such as HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 Desalting columns can be used, see Ordering information.*

6 Optimization

The protocols recommended in this instruction should be regarded as starting points for purification of histidine-tagged proteins. Some conditions may require optimization.

Examples of conditions which may require optimization are:

- sample loading flow rate
- sample volume
- imidazole concentration during wash
- wash flow rate
- wash volume
- temperature
- buffer composition, pH etc.

For more information, see Section *Buffer preparation*.

7 Cleaning-in-place

When an increase in back pressure is seen, the column should be cleaned. Different methods for cleaning-in-place (CIP) are described in the table below. Do not exceed a flow velocity of 150 cm/h during CIP.

To remove...	Then...
Ionically bound proteins	1 Wash with several column volumes (CV) of 1.5 M NaCl. 2 Wash with approximately 10 CV distilled water or equilibration buffer.
Precipitated proteins, hydrophobically bound proteins, and lipoproteins	1 Wash the column with 1 M NaOH, contact time usually 1 to 2 h (12 to 24 h for endotoxin removal). 2 Wash with approximately 10 CV equilibration buffer.
Hydrophobically bound proteins, lipoproteins, and lipids.	1 Wash with 5 to 10 CV 30% isopropanol for about 15 to 20 min. 2 Wash with approximately 10 CV distilled water or equilibration buffer. OR 1 Wash with 2 CV detergent in a basic or acidic solution. Use, for example, 0.1 to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 h. 2 Remove residual detergent by washing with at least 5 CV 70% ethanol. 3 Wash with approximately 10 CV equilibration buffer.

8 Storage

Store Ni Sepharose excel and HisTrap excel in 20% ethanol at 4°C to 30°C. Do not freeze.

Make sure that the HisTrap excel column is tightly sealed to avoid drying out.

9 Tips and hints

The following tips may be of assistance.
For further questions about Ni Sepharose excel or HisTrap excel, please visit www.gelifesciences.com/protein-purification, refer to the Data file (29-0168-49), or contact our technical support or your local representative.

Issue	**Possible cause** ***Suggested action***
Increased back pressure	• Freeze-thawing of the sample may increase precipitation and aggregation. *Avoid freeze-thawing.* *Remove precipitate.* • Increased sample viscosity. *If the purification has been performed in a cold room, move to room temperature if possible. Alternatively, decrease flow rate during sample loading.*
Column has clogged	• High content of particulate material in the sample. *Clean the column according to Section Cleaning-in-place. If column cleaning is unsuccessful, replace the column.* *Optimize sample pre-treatment before the next sample loading.*
Compression of the gel in manually packed columns	• High viscosity, too high flow rate. *Re-adjust the top adapter.*
No histidine-tagged protein in the purified fractions	• No histidine-tagged protein present in the starting material. *Verify presence of histidine-tagged protein in the starting material, for example by Western blotting.* • Protein found in the flowthrough: The histidine-tag is not sufficiently exposed. *Consider using another position for the histidine-tag.* • Protein found in the flowthrough: Buffer/sample composition is not optimal. *Check pH and composition of sample and equilibration buffer. Make sure that the concentration of imidazole is not too high. Note that the use of imidazole in equilibration buffer and sample is not recommended.*

Issue	Possible cause *Suggested action*
No histidine-tagged protein in the purified fractions (cont.)	• Protein eluted during wash. *Ensure that the concentration of imidazole is not too high.* • Elution conditions are too mild (histidine-tagged protein still bound). *Elute with increased imidazole concentration.* • Protein has precipitated in the column. *Decrease amount of sample, or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Try detergents or change NaCl concentration.* • Nonspecific hydrophobic, ionic or other interaction prevents elution. *Add for example a nonionic detergent to the elution buffer or change the NaCl concentration.*
The eluted protein is not sufficiently pure (multiple bands on SDS polyacrylamide gel)	• Partial degradation of tagged protein by proteases. *Add protease inhibitors.* • Contaminants have high affinity for nickel ions. *Elute with a stepwise or linear imidazole gradient to determine optimal imidazole concentrations to use for wash. Increase the wash length before elution and/or the imidazole concentration during wash. A shallow imidazole gradient (20 column volumes or more) may separate proteins with similar binding strengths. If optimized conditions do not remove contaminants, adding a second chromatography step such as gel filtration may be necessary.* • Contaminants are associated with the tagged protein. *Add detergent or glycerol to the wash buffer to disrupt non-specific interactions.* • Electrostatic interactions of contaminants with the tagged protein or the chromatography medium. *Increase the sodium chloride concentration to 1 to 2 M in buffers.*

Issue	Possible cause *Suggested action*
Histidine-tagged protein is eluted too early during elution	• When eluting using imidazole, an initial, transient pH drop may be observed. The lower pH may affect the affinity for bound proteins negatively, and a partially premature elution may be observed for loosely bound proteins. *Increase the buffering capacity of the wash and elution buffers, which mitigates imidazole's effect on pH.*
Inadequate binding of histidine-tagged protein during sample loading/wash	• No histidine-tagged protein present in the starting material. *Verify presence of histidine-tagged protein in the starting material, for example by Western blotting.* • The histidine-tag is not sufficiently exposed. *Consider using another position for the histidine-tag.* • Buffer/sample composition is not optimal. *Check pH and composition of sample, equilibration buffer and wash buffer. Make sure that the concentration of imidazole is not too high.* • Column capacity is exceeded. *Change to a larger column. For example, if HisTrap excel 1 ml column has been used, change to HisTrap excel 5 ml.* ***Note:*** *Ni Sepharose excel is mainly intended for IMAC purification of histidine-tagged proteins secreted into cell culture supernatants from eukaryotic cells, such as insect cells or CHO cells. It is also suitable for purification of histidine-tagged proteins originating from other samples causing nickel stripping. When purifying proteins from other types of samples, use Ni Sepharose 6 Fast Flow or Ni Sepharose High Performance.*
Temperature-related differences between optimization and actual purification	• Temperature affects column properties. *If optimization is performed, always do this at the temperature intended for the actual purification.*

10 Ordering information

Product	Quantity	Code No.
Ni Sepharose excel	25 ml	17-3712-01
	100 ml	17-3712-02
	500 ml	17-3712-03
HisTrap excel	1 × 1 ml	29-0485-86
	5 × 1 ml	17-3712-05
	5 × 5 ml	17-3712-06

Related products	Quantity	Code No.
His Mag Sepharose excel	2 × 1 ml	17-3712-20
	5 × 1 ml	17-3712-21
	10 × 1 ml	17-3712-22
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml	17-5318-03
HisTrap FF crude	1 × 1 ml	29-0486-31
	5 × 1 ml	11-0004-58
	100 × 1 ml [1]	11-0004-59
	5 × 5 ml	17-5286-01
	100 × 5 ml[1]	17-5286-02
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	11-0003-29
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01

[1] Special pack size delivered on specific customer order

Empty lab-scale columns	Code No.
Tricorn 10/20 column, 10 mm i.d.	28-4064-13
Tricorn 10/50 column, 10 mm i.d.	28-4064-14
Tricorn 10/100 column, 10 mm i.d.	28-4064-15
XK 16/20 column, 16 mm i.d.	28-9889-37
XK 16/40 column, 16 mm i.d	28-9889-38
XK 26/20 column, 26 mm i.d.	28-9889-48
XK 26/40 column, 26 mm i.d.	28-9889-49
XK 50/20 column, 50 mm i.d.	28-9889-52
XK 50/30 column, 50 mm i.d.	28-9889-53
HiScale 16/20 column, 16 mm i.d.	28-9644-41
HiScale 16/40 column, 16 mm i.d.	28-9644-24
HiScale 26/20 column, 26 mm i.d.	28-9645-14
HiScale 26/40 column, 26 mm i.d.	28-9645-13
HiScale 50/20 column, 50 mm i.d.	28-9644-45
HiScale 50/40 column, 50 mm i.d.	28-9644-44

HiTrap accessories	Quantity	Code No.
1/16" male/luer female [1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	5	18-3858-01
Union luerlock female/M6 female[1]	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16"	2	11-0004-64
Fingertight stop plug, 1/16" [2]	5	11-0003-55

[1] One connector included in each HiTrap package.

[2] One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media Selection Guide	18-1121-86
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78
Data file, His Mag Sepharose excel, Ni Sepharose excel, HisTrap excel	29-0168-49

For local office contact information, visit
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