Pierce™ In-Gel Tryptic Digestion Kit

Catalog Number 89871

Doc. Part No. 2161468 Pub. No. MAN0011497 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

In-gel digestion coupled with mass spectrometric analysis is a powerful tool for the identification and characterization of proteins (Lahm and Langen, 2000; Patterson and Aebersold, 2003). The Pierce™ In-Gel Tryptic Digestion Kit provides a complete set of reagents to perform approximately 150 digestions on colloidal coomassie or fluorescent dye-stained protein bands. The kit includes Thermo Scientific™ Pierce™ Trypsin Protease, MS Grade, destaining buffers, digestion buffers, reduction reagents, and alkylation reagents. The methodology of this kit has been designed to function with a wide range of protein band concentrations producing complete and accurate digest for dependable mass spectrometric (MS) analysis.

Contents and storage

The Pierce™ In-Gel Tryptic Digestion Kit contains sufficient reagents for approximately 150 in-gel digestions.

Item	Amount	Storage[1]
Pierce™ Trypsin Protease, MS Grade	20 µg	–20°C
Trypsin Storage Solution	40 µL	4°C
Acetonitrile	3 x 24 mL	
Ammonium Bicarbonate	300 mg	
TCEP (Tris[2-carboxyethyl]phosphine)	500 µL	
Iodoacetamide (IAA)	500 mg	

[1] For additional storage and handling information for each component upon receipt, see “Before you begin” on page 3.

Workflow

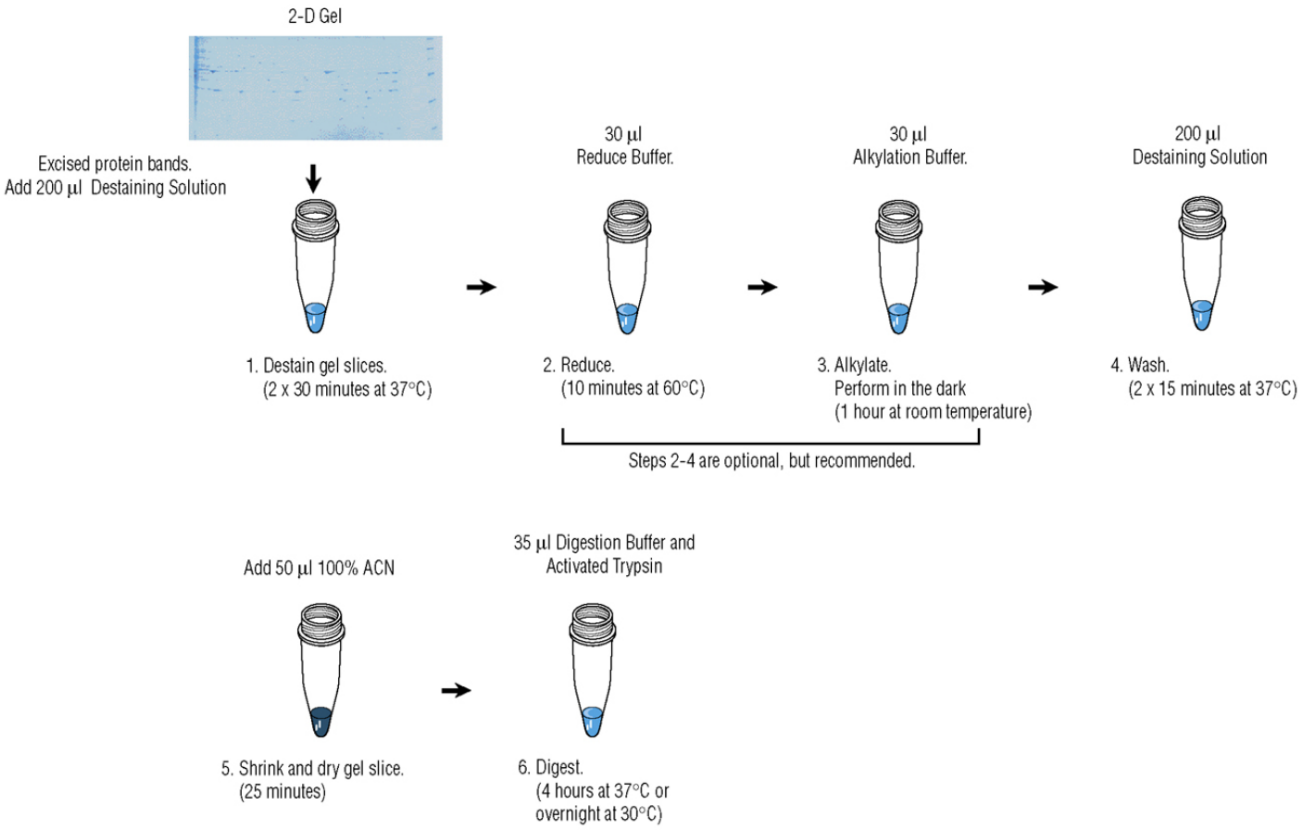

Figure 1 Pierce™ In-Gel Tryptic Digestion Kit procedure summary.

Additional information

- Trypsin is a serine protease that specifically cleaves peptide bonds at the carboxyl side of lysine and arginine residues. However, cleavage can be blocked or slowed by a proximal acidic, aromatic, or proline residue; proline having the most significant effect. Peptide fragments with one missed cut are common and should be taken into consideration during mass analysis.
- The Pierce™ Trypsin Protease, MS Grade provided in this kit displays only limited autolytic activity that should not interfere with mass spectral analysis. A trypsin fragment of mass 842.51 (m/z, M + H) will be the most common using standard conditions and can be used as an internal standard.
- The Pierce™ In-Gel Tryptic Digestion Kit is designed for collodial coomassie or fluorescent dye-stained acrylamide gel slices. For protein bands stained with mass spectrometry-compatible silver stains or reversible zinc staining (Cat. No. 24582), alternative destaining procedures will be required (Shevchenko et al., 1996; Shevchenko and Shevchenko, 2001).
- For SDS-PAGE separations, use polyacrylamide gels of 1 mm thickness. Gels of other thicknesses may result in reduced peptide recovery yield (Speicher et al., 2000).
- Reduction and alkylation of cystine residues using TCEP and IAA, respectively, improves the recovery of cystine-containing peptides from in-gel digests and minimizes the appearance of unknown masses in MS analysis from disulfide bond formation and side chain modification. Alkylation is optional, but highly recommended (Sechl and Chalt,1998). A reliable and optimized method for reduction and alkylation, as part of the in-gel digestion protocol, is provided (see “(Optional) Reduce and alkylate sample” on page 4). Nevertheless, alkylation can be preformed in a variety of ways dependent on the application (Herbert et al., 2001; Galvani et al., 2001; Galvani et al., 2001), and no one method is optimal for all applications.

  Note: Alkylation with iodoacetamide increases the mass of a peptide by 57.02 for each cystine present. Acrylamide modification of cystine results in a peptide mass increase of 71.04.

  Note: When separating and examining proteins by 2D gel electrophoresis using alkaline conditions (i.e., pH >8), alkylate the sample before isoelectric focusing (IEF). The use of an alternative reducing agent (e.g., hydroxyethyl disulfide) may help to avoid spurious banding in the alkaline regions caused by disulfide bond formation (Herbert et al., 2001; Olsson et al., 2002). Alkylation of the sample before 2D electrophoresis is not required for proteins with a pI <8.0.

Required materials not supplied

- 600 µL microcentrifuge tubes
- 50 mL capped bottle or equivalent
- 10 mL storage bottle, tube, or equivalent
- Ultrapure water [18 megaohm (MΩ) equivalent]

  Note: Use ultrapure water in the preparation of all materials.
- Compatible protein gel stain:
  - Pierce™ Zinc Reversible Stain Kit (Cat. No. 24582)
  - GelCode™ Blue Stain Reagent (Cat. No. 24590)
  - Pierce™ Silver Stain for Mass Spectrometry (Cat. No. 24600)

Before you begin

Note: Some of the solutions required for the Pierce™ In-Gel Tryptic Digestion Kit require occasional preparation while others should be prepared just before use as needed; therefore, plan accordingly.

1 Prepare trypsin stock

Pierce™ Trypsin Protease, MS Grade (20 µg) is supplied lyophilized and may be stored in this form at –20°C for >1 year without significant loss in activity.

1. When required, prepare the trypsin stock solution by hydrating the lyophilized trypsin with 20 µL of the supplied Trypsin Storage Solution. This solution contains components that inactivate and protect the enzyme from autodigestion.
2. To minimize freeze/thaw cycles and to increase storage stability, divide the hydrated trypsin into 4 separate tubes of approximately 5 µL each. Store each aliquot at –20°C in a nonfrost-free freezer. This solution is used to form the trypsin working solution as needed (see “Prepare trypsin working solution” on page 3).

2 Prepare trypsin working solution

1. When required, thaw a trypsin stock aliquot on ice.
2. Dilute the stock 10-fold by adding 45 µL of ultrapure water.

   This solution may be stored at –20°C for 2 months without significant activity loss.

3 Prepare destaining solution

Mix 80 mg of ammonium bicarbonate with 20 mL of acetonitrile (ACN) and 20 mL of ultrapure water.

The destaining solution may be stored at 4°C for 2 months. This stock solution is sufficient for 50–100 digestions and can be prepared 3 times with this kit.

4 Prepare digestion buffer

Mix 10 mg of ammonium bicarbonate with 5 mL of ultrapure water (final concentration approximately 25 mM).

The digestion buffer may be stored at 4°C for 2 months. This stock solution can be prepared 3 times with this kit.

Note: An excess of digestion buffer is supplied to minimize the need for long-term storage and weighing minute quantities of ammonium bicarbonate.

5 Prepare reducing buffer

Prepare just before use (see step 2.1).

Mix 3.3 µL of TCEP with 30 µL of digestion buffer for each digest to be performed.

Final TCEP concentration is approximately 50 mM.

Note: Do not store the reducing buffer.

6 Prepare alkylation buffer

Prepare just before use in foil-wrapped tubes to avoid exposure to light (see step 2.3).

6 Prepare alkylation buffer *(continued)*

1. To avoid weighing sub-microgram quantities of IAA when a small number of samples are being processed, dissolve 7 mg of IAA in 70 µL water to make a 5X stock (approximately 500 mM final concentration).
2. Dilute 7 µL of the 5X stock solution with 28 µL of digestion buffer for each digest being performed to make the final alkylation buffer.

   If greater than 10 samples are being digested simultaneously, increase the volume of stock accordingly. Excess IAA has been supplied with this kit.

   Note: Do not store the alkylation buffer or stock solution.

7 **Prepare activated trypsin**

Shortly before use, dilute 1 µL of trypsin working solution with 9 µL of digestion buffer for each sample being processed (see step 3.3).

Final concentration will be approximately 10 ng/µL. Keep activated trypsin on ice until use.

Note: Do not store activated trypsin.

Note: The recommended amount of trypsin used per digest is 100 ng. This amount of trypsin can be reliably used for a wide variety of protein concentration within an excised gel band. However, if the protein band contains significantly less than approximately 20 ng protein (300 fmol), 25 ng of trypsin may be used per digest by diluting the trypsin working solution an additional 4-fold with digestion buffer.

Digest slices from 1D or 2D gel electrophoresis separated proteins

1 **Prepare and destain bands**

Note: This procedure is for collodial coomassie or fluorescent dye-stained acrylamide gel slices. Alternative destaining procedures are required for silver- or zinc-stained protein bands. See “Required materials not supplied” on page 3 for a listing of compatible protein stains and “Additional information” on page 2 for alternative destaining procedures.

1. Use a spot picker or scalpel to excise the protein band of interest from the 1D or 2D gel. Cut the band into 1 mm x 1 mm to 2 mm x 2 mm pieces. Place the pieces into a 600 µL receiver tube.

   Note: Take care to include only the stained region of the gel.
2. Add 200 µL of destaining solution to the gel pieces. Incubate the sample at 37°C for 30 minutes with shaking.
3. Remove and discard the destaining solution from the tube.
4. Repeat step 1.2 to step 1.3.
5. Proceed to step 2.1 if reduction and alkylation are required. If reduction and alkylation are not required, proceed directly to step 3.1.

2 ***(Optional)* Reduce and alkylate sample**

Note: Reduction and alkylation are optional but recommended if high-sequence coverage is desired. If the sample is reduced and alkylated before or during electrophoresis, it may be possible to omit these steps without affecting results. However, alkylation is inhibited or slowed by a variety of conditions, such as the presence of thiourea, SDS, or a pH <7.0; therefore, alkylation of the sample before electrophoresis may not be complete.

1. Prepare the reducing buffer as described in “Prepare reducing buffer” on page 3. Add 30 µL of reducing buffer to the tube containing the sample and incubate at 60°C for 10 minutes.
2. Allow samples to cool; then remove and discard the reducing buffer from the tube.
3. Prepare the alkylation buffer as described in “Prepare alkylation buffer” on page 3. Add 30 µL of alkylation buffer to the tube. Incubate the sample in the dark at room temperature for 1 hour.
4. Remove and discard the alkylation buffer from the tube. Wash the sample by adding 200 µL of destaining buffer to the tube. Incubate the sample at 37°C for 15 minutes with shaking.

2 *(Optional)* Reduce and alkylate sample *(continued)*

5. Remove and discard the destaining buffer from the tube.
6. Repeat step 2.4 to step 2.5.
7. Proceed to step 3.1.

3 **Digest gel slices**

1. Shrink gel pieces by adding 50 µL of acetonitrile. Incubate the sample for 15 minutes at room temperature.
2. Carefully remove the acetonitrile and allow gel pieces to air dry for 5–10 minutes.
3. Prepare activated trypsin as described in "Prepare activated trypsin" on page 4. Swell gel pieces by adding 10 µL of the activated trypsin solution to the tube. Incubate the sample at room temperature for 15 minutes.

   Note: If 10 µL is insufficient to cover and fully swell gel pieces, increase the volume accordingly.
4. Add 25 µL of digestion buffer to the tube. Incubate the sample at 37°C for 4 hours or at 30°C overnight with shaking.
5. Remove the digestion mixture and place in a clean tube.
6. *(Optional)* To further extract peptides, add 10 µL of 1% trifluoroacetic acid or 1% formic acid solution to the gel pieces and incubate for 5 minutes. Remove the extraction solution and add to the digestion mixture (step 3.5). This step also serves to inactivate trypsin, stopping additional enzymatic activity. A second extraction generally results in only a minor increase in peptide recovery.
7. The sample is now ready for liquid chromatographic separation and electrospray ionization mass spectrometry (LC-ESI MS) or for additional processing/clean-up as required for matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) or nanospray ionization mass spectrometry (see Pierce™ C18 Spin Columns, Cat. No. 89870).

   Note: To prevent clogging or column damage, ensure the sample is free of any acrylamide pieces before applying to a LC-ESI MC system.

Troubleshooting

Observation	Possible cause	Recommended action
Incomplete digestion	Insufficient enzymatic activity.	Increase incubation time.
		Ensure gel slice was dry before addition of enzyme to pull trypsin into gel slice and increase hydration volume.
	Enzyme was losing activity.	Use a new trypsin stock aliquot.
	Incorrect pH.	Ensure gel slice has been completely destained and trypsin working solution has been diluted with digestion buffer.
	Residual SDS.	Ensure gel slice has been completely destained.
Poor mass spectrum	Concentration or detection limits of application.	Ensure sample is within the detection limit of the specific downstream application; concentrate digest on C18 sample prep device (Cat. No. 89870). **Note:** Limits vary considerably based on application and instrumentation.
	Interfering agents.	Clean up digest with C18 sample prep device.

References

Lahm HW, Langen H. (2000) Mass spectrometry: A tool for the identification of proteins separated by gels. *Electrophoesis* 21:2105–2114.

Patterson SD, Aebersold R. (2003) Proteomics: the first decade and beyond. *Nat Genet* 33 supplement:311–323.

Shevchenko A et al. (1996) Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem* 68:850–858.

Shevchenko A, Shevchenko A. (2001) Evaluation of the efficiency of in-gel digestion of proteins by peptide isotopic labeling and MALDI mass spectrometry. *Anal Biochem* 296:279–283.

Speicher KD et al. (2000) Systematic analysis of peptide recoveries from in-gel digestions for protein identifications in proteome studies. *J Biomolecular Techniques* 11:74–86.

Sechl S, Chalt BT. (1998) Modification of cysteine residues by alkylation. A tool in peptide mapping and protein identification. *Anal Chem* 70:5150–5158.

Herbert B et al. (2001) Reduction and alkylation of proteins in preparation of two-dimensional map analysis: Why, when, and how? *Electrophoresis* 22:2046–2057.

Galvani M et al. (2001) Alkylation kinetics of proteins in preparation for two-dimensional maps: A matrix assisted desorption/ionization-time of flight-mass spectrometry investigation. *Electrophoresis* 22:2058-65.

Galvani M et al. (2001) Protein alkylation in the presence/absence of thiourea in proteome analysis: A matrix assisted desorption/ionization-time of flight-mass spectrometry investigation. *Electrophoresis* 22:2066–2074.

Olsson I et al. (2002) Organic disulfides as a means to generate streak-free two-dimensional maps with narrow range basic immobilized pH gradient strips as first dimension. *Proteomics* 2:1630–1632.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011497

Revision	Date	Description
B.0	4 November 2022	The format and content were updated.
A.0	17 October 2015	New document for the Pierce™ In-Gel Tryptic Digestion Kit.