

## Are you missing important peaks?

Get better Monoclonal Antibody Resolution  
with MAbPac RP columns for High Performance  
Reverse Phase Chromatography

# MABPac RP Column

The Thermo Scientific™ MABPac™ RP columns are designed for comprehensive characterization of mAb purity by reversed-phase (RP) liquid chromatography. High resolution of intact mAb, mAb fragments such as light chain (LC), heavy chain (HC), Fc and Fab, scFc and F(ab')<sub>2</sub>, will reveal the location, as well as nature, of modification using LC/UV or LC/MS.

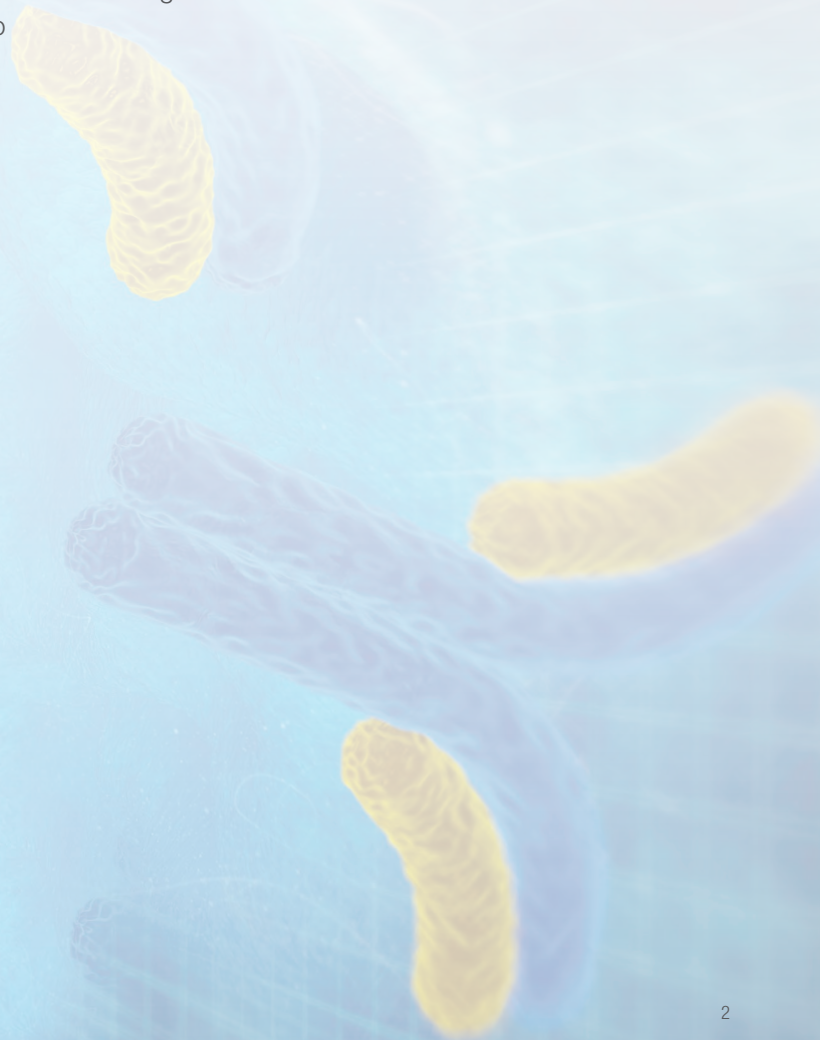


## Product Highlights

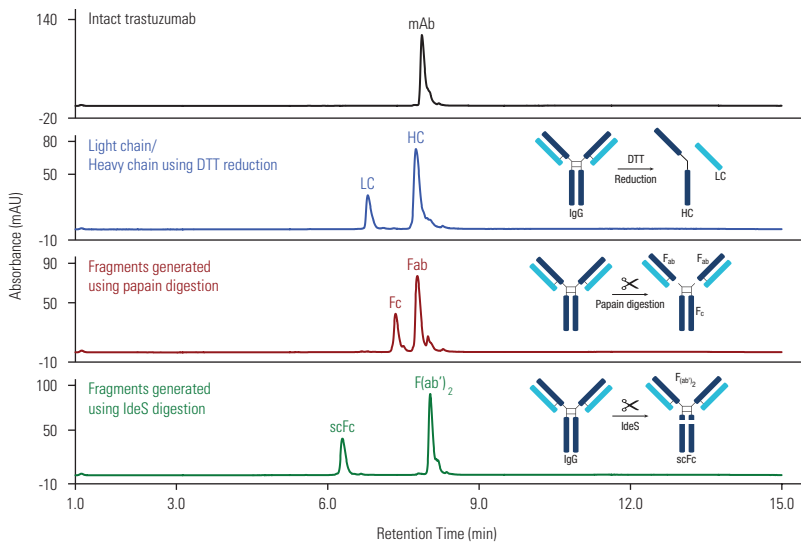
- Superior resolution of monoclonal antibodies and related substances: unique macroporous resin with faster mass transfer capabilities
- High efficiency columns with lower carry-over than silica based columns – engineered into the surface chemistry
- Excellent MS compatibility
- Wider operating pH range (0–14) easy cleaning with up to 800 mM NaOH (not possible with silica based columns)
- High temperature stability (up to 110 °C) for better chromatographic separation
- Fast separations capability for high throughput separations
- Wide loadability range with 3 orders of magnitude: 0.2 ng to 20 µg

## Optimized Column Technology

The MABPac RP column is a reversed-phase (RP) column based on a macroporous resin specifically designed for high resolution separation of intact monoclonal antibodies (mAbs) and mAb fragments. The stationary phase is fully compatible with mass spectrometry friendly organic solvents such as acetonitrile and isopropanol, as well as low pH eluents containing trifluoroacetic acid or formic acid.



# Fast Separation of Intact Proteins/mAbs and Fragments



Column: MAbPac RP, 4  $\mu$ m  
 Format: 3  $\times$  50 mm  
 Mobile phase A: H<sub>2</sub>O/FA/TFA (99.88 : 0.1:0.02 v/v/v)  
 Mobile phase B: MeCN/ H<sub>2</sub>O/FA/TFA (90: 9.88 : 0.1:0.02 v/v/v/v/v)  
 Gradient:

| Time (min) | %A | %B |
|------------|----|----|
| 0.0        | 80 | 20 |
| 1.0        | 80 | 20 |
| 11.0       | 55 | 45 |
| 12.0       | 55 | 45 |
| 14.0       | 80 | 20 |
| 15.0       | 80 | 20 |

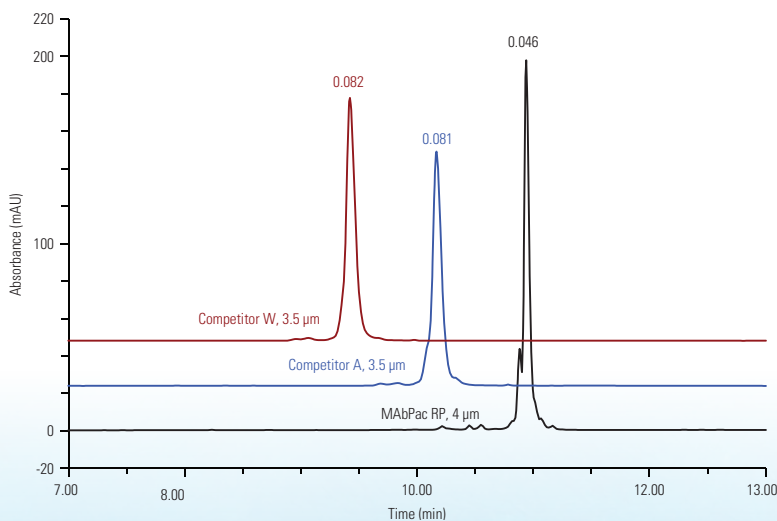
Temperature: 80 °C  
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 5  $\mu$ L  
 Detection: UV (280 nm)

Sample:  
 (a) Trastuzumab (5 mg/mL)  
 (b) Trastuzumab + DTT (4 mg/mL)  
 (c) Trastuzumab + Papain (2 mg/mL)  
 (d) Trastuzumab + IdeS (2 mg/mL)

Figure 1. Separation of mAb and mAb fragments.

## Superior Separation of mAb

MAbPac RP chemistry and morphology provides superior resolution of intact mAb and variants, as shown in this comparison with other column suppliers. Twice as many variants, with almost half of the peak width half height, are clearly resolved using the MAbPac RP column: resulting in easier and better identification throughout the analysis (Figure 2).



Column: RP columns  
 Format: 3  $\times$  50 mm or 4.6  $\times$  50 mm  
 Mobile phase A: H<sub>2</sub>O/TFA (99.9 : 0.1 v/v)  
 Mobile phase B: MeCN/ H<sub>2</sub>O/TFA (90: 9.9 : 0.1 v/v/v)  
 Gradient:

| Time (min) | %A | %B |
|------------|----|----|
| -3.0       | 80 | 20 |
| 1.0        | 80 | 20 |
| 11.0       | 50 | 50 |
| 12.0       | 50 | 50 |
| 12.1       | 80 | 20 |
| 15.0       | 80 | 20 |

Temperature: 80 °C  
 Flow Rate: 0.5 mL/min or 1.0 mL/min  
 Inj. Volume: 0.5  $\mu$ L or 1  $\mu$ L  
 Detection: UV (280 nm)

Sample: NISTmAb (10.0 mg/mL)  
 Peak Label: PWHH (min)

Figure 2. Comparison of NISTmAb separation.

# LC/MS Analysis of Intact mAb and mAb Fragments

The MAbPac RP column can be directly coupled to the mass spectrometer for MS detection of mAb and mAb fragments. Combination with the Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer enables accurate mass measurement of mAbs (Figure 3).

Column: MAbPac RP, 4 μm  
 Format: 3 × 50 mm  
 Mobile phase A: H<sub>2</sub>O/FA/TFA (99.88 : 0.1:0.02 v/v/v)  
 Mobile phase B: MeCN/ H<sub>2</sub>O/FA/TFA (90: 9.88 : 0.1:0.02 v/v/v/v)  
 Gradient:
 

| Time (min) | %A | %B |
|------------|----|----|
| 0.0        | 80 | 20 |
| 1.0        | 80 | 20 |
| 11.0       | 55 | 45 |
| 12.0       | 55 | 45 |
| 14.0       | 80 | 20 |
| 15.0       | 80 | 20 |

Temperature: 80 °C  
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 1 μL  
 UV Detection: 280 nm  
 MS Detection: Positive-ion mode  
 Mass Spec: Q ExactivePlus

Sample: Reduced trastuzumab (4 mg/mL)

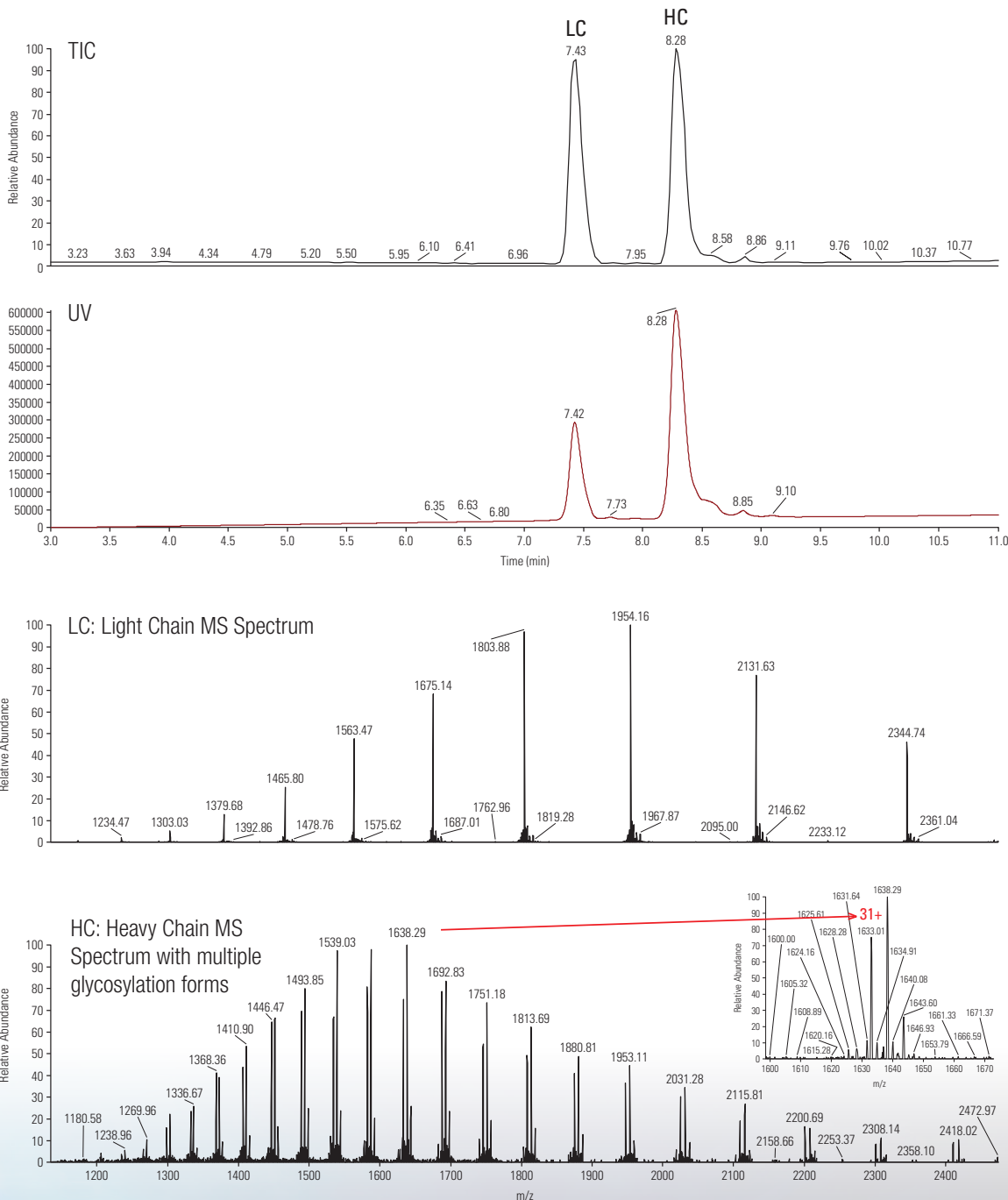
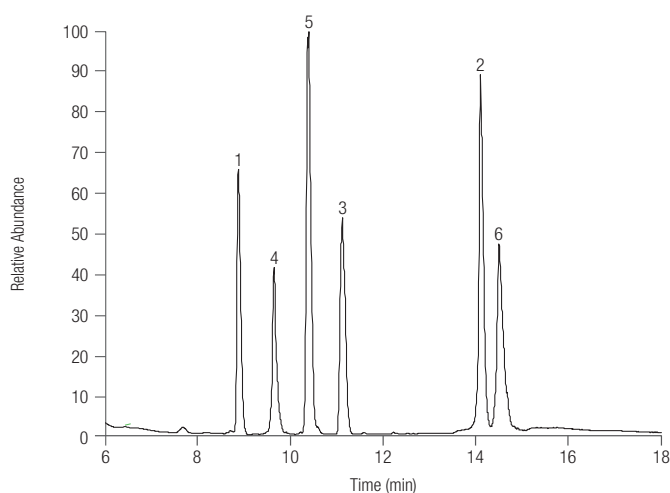


Figure 3. Reduced mAb LC/MS analysis.

# Top-down LC/MS/MS Analysis of Intact Protein Standards

Thermo Scientific™ Pierce™ Intact Protein Mix consists of six recombinant proteins: IGF-I LR3 (9 kDa), thioredoxin (12 kDa), protein G (21 kDa), carbonic anhydrase II (29 kDa), protein AF (51 kDa), and exo klenow (68 kDa). These proteins are selected to satisfy the following criteria: 1) evenly covering a MW range of 10kD–66kD, 2) presenting mostly clean, modification and adduct-free ESI spectra, and 3) having ESI charge state distributions covered a wide  $m/z$  range from 500–2,000. A 1 mm ID MAbPac RP column was chosen to analyze these proteins because it provides higher sensitivity than larger internal diameter columns, when sample size is limited.

Figure 4a shows baseline separation of all six proteins using an acetonitrile/H<sub>2</sub>O/formic acid mobile phase. Top-down MS/MS spectra were acquired on a Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer using top 3–5 DDA method. OT MS1 data was acquired at resolution settings of 15 at  $m/z$  200 and OTMS2 at a resolution of 120K at  $m/z$  200. Figure 4b shows deconvolution results from Thermo Scientific™ Protein Deconvolution™ 4.0 software and Figure 4c shows top-down results from ProSight PD 1.1 node in Proteome Discoverer 2.1 software.



Column: MAbPac RP, 4  $\mu$ m  
 Format: 1.0  $\times$  150 mm  
 Mobile phase A: H<sub>2</sub>O/FA (99.9 : 0.1 v/v)  
 Mobile phase B: MeCN/FA (99.0: 0.1 v/v)  
 Gradient: Time (min) %A %B  
 0.0 90 10  
 1.0 80 20  
 15.0 55 45  
 16.0 10 90  
 20.0 10 90  
 20.1 90 10  
 25.0 90 10  
 Temperature: 60 °C  
 Flow Rate: 0.1 mL/min  
 Inj. Volume: 1  $\mu$ L  
 MS Detection: positive-ion mode  
 Mass Spec: Q Exactive HF  
 Sample: Pierce Intact Protein Standard Mix, 500 ng/ $\mu$ L (P/N A35526)  
 1. IGF-I LR3 (9 kDa)  
 2. Thioredoxin (12 kDa)  
 3. Protein G (21 kDa)  
 4. Carbonic Anhydrase II (29 kDa)  
 5. Protein AF (51 kDa)  
 6. Exo Klenow (68 kDa)

Figure 4a. Total ion chromatogram of the LC/MS analysis of the Intact Protein Standard Mix.

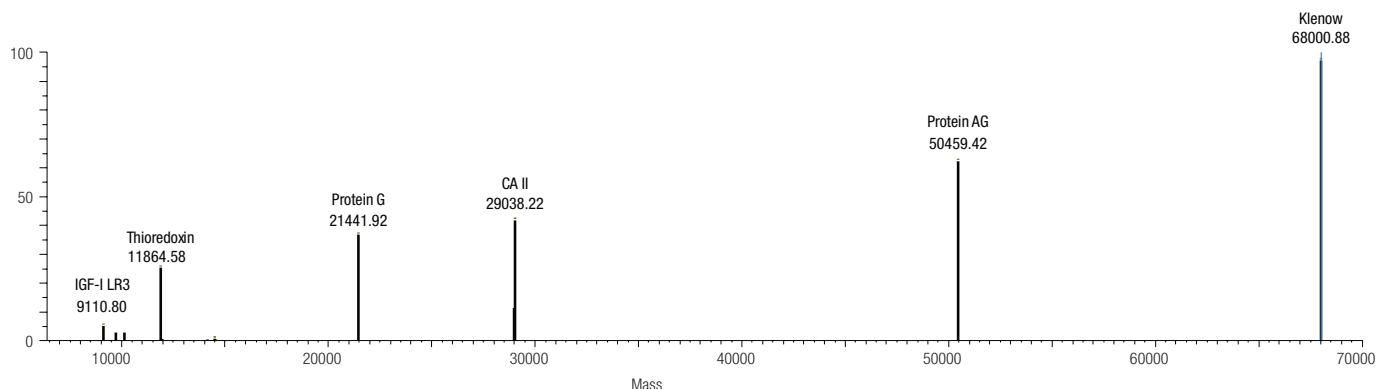


Figure 4b. Average masses for all six proteins in the Intact Protein Standard Mix.

|   | Checked                             | Master                              | Accession | Description                                  | Coverage | # Peptides | # PSMs | # Unique Peptides | # Protein Groups | # AAs | MW [kDa] | calc. pt | # Peptides ProSightPD Absolute Mass Search |
|---|-------------------------------------|-------------------------------------|-----------|----------------------------------------------|----------|------------|--------|-------------------|------------------|-------|----------|----------|--------------------------------------------|
| 1 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | ProteinG  | ProteinG Immunoglobulin G-binding protein G  | 100%     | 2          | 17     | 2                 | 1                | 198   | 21.4     | 4.75     | 2                                          |
| 2 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | rCA       | rCA Carbonic anhydrase 2                     | 100%     | 1          | 9      | 1                 | 1                | 260   | 29.1     | 7.12     | 1                                          |
| 3 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | M_trx     | Thioredoxin, mitochondrial                   | 99%      | 1          | 2      | 1                 | 1                | 108   | 12.0     | 5.08     | 1                                          |
| 4 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | ProteinAG | ProteinAG Immunoglobulin G-binding protein A | 100%     | 1          | 8      | 1                 | 1                | 455   | 50.6     | 4.77     | 1                                          |
| 5 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | IGF_LR3   | Insulin-like growth factor I                 | 100%     | 1          | 2      | 1                 | 1                | 83    | 9.1      | 8.28     | 1                                          |
| 6 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | Klenow    | Klenow DNA polymerase [ECO.0000256]          | 100%     | 1          | 4      | 1                 | 1                | 605   | 68.0     | 6.11     | 1                                          |

Figure 4c. List of identified proteins using Proteome Discoverer 2.1 software.

## Physical Data

|                   |                                                                                        |
|-------------------|----------------------------------------------------------------------------------------|
| Chemistry         | Phenyl                                                                                 |
| Polymer substrate | DVB particles                                                                          |
| Particle size     | 4 $\mu\text{m}$                                                                        |
| Pore size         | 1,500 $\text{\AA}$                                                                     |
| Column housing    | Stainless steel for the 3.0 and 2.1 mm ID<br>Glass Lined Stainless Steel for 1.0 mm ID |

## Operational Specifications

| Column    | Column ID (mm) | Flow Rate (mL/min) | Pressure Limit (psi) | Temperature ( $^{\circ}\text{C}$ ) | pH Range |
|-----------|----------------|--------------------|----------------------|------------------------------------|----------|
| MABPac RP | 3.0            | 0.50–1.00          | 4,000                | < 110                              | 0–14     |
| MABPac RP | 2.1            | 0.30–0.60          | 4,000                | < 110                              | 0–14     |
| MABPac RP | 1.0            | 0.075–0.15         | 4,000                | < 110                              | 1–7      |

## Ordering Information

| Description       |                          | Particle Size ( $\mu\text{m}$ ) | Length (mm) | 1.0 mm ID | 2.1 mm ID | 3.0 mm ID |
|-------------------|--------------------------|---------------------------------|-------------|-----------|-----------|-----------|
| MABPac RP Columns | Analytical               | 4                               | 50          | 303182    | 088648    | 088645    |
|                   |                          |                                 | 100         | 303183    | 088647    | 088644    |
|                   |                          |                                 | 150         | 303184    | 303270    | 303269    |
|                   | Guard Cartridges (2/pk)* |                                 | 10          |           | 088649    | 088646    |

\* Standard Guard Cartridge Holder Required: P/N 069580

Find out more at [thermofisher.com/mabpac](https://thermofisher.com/mabpac)

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