

Protein assay compatibility table

TR0068.5

Introduction

No single protein assay method is compatible with all sample components that might be encountered in typical laboratory research. To alleviate the frustration of discovering these incompatibilities by trial and error, our technical support scientists have characterized the effects of commonly used buffers and sample components on the behavior of seven different Thermo Scientific Pierce* Protein Assays.

Results of these compatibility experiments are summarized in tabular form on the following two pages. The table serves as a guide for assessing which protein assay(s) might be most effective with a given sample type. Use this information as a guide only. It is the researcher's responsibility to validate the compatibility of specific sample buffers. Even minor changes in buffers and components affect each assay method to some extent. Researchers must decide if these effects are acceptable for their specific purposes.

About the Compatibility Table

The table indicates the maximum compatible concentrations for substances tested with the *Test Tube* procedure on the midpoint concentration of BSA standard (i.e., 1000µg/mL for most assays; 10µg/mL for the Micro BCA Assay). Substances were considered compatible at the indicated concentration if the error in protein concentration estimation caused by the presence of the substance in the sample was less than or equal to 10%.

Tested Thermo Scientific Pierce Protein Assays.

| Part No. | Name |
|----------|---|
| 22662 | 660 nm |
| 23225 | BCA |
| 23235 | Micro BCA |
| 23250 | BCA – Reducing Agent Compatible (BCA-RAC) |
| 23252 | Microplate BCA-RAC |
| 23240 | Modified Lowry |
| 23236 | Coomassie Plus (Bradford) |
| 23200 | Coomassie (Bradford) |

Notes and Symbols:

- Compounds are listed alphabetically using common names or abbreviations, except sodium compounds which are alphabetized under “Na”.
- Dilutions are expressed as “neet” (= undiluted) or in the form of a ratio, where “1:2” means 2-fold dilution.
- n/a Denotes that the compound was not tested in this assay.
- Ø Denotes compounds that were not compatible at the lowest concentration tested.
- * Thermo Scientific product trademark; “PER Reagents” are Thermo Scientific Pierce Cell Lysis Reagents.
- ** Trademarks that are the property of other companies; see end of document.
- † Value when the 660nm Assay is run using the ionic detergent compatibility reagent (IDCR, Part No. 22663).
- †† Selected values for the regular BCA-RAC Kit are given in parentheses in the column for the Microplate BCA-RAC.
- ‡ Compound (buffer) whose formulation is described more fully in the following table:

| Part No. | Buffer | Formulation |
|----------|---------------------------------|---|
| - | 2-D sample buffer | (8M urea, 4% CHAPS) or (7M urea, 2M thiourea, 4% CHAPS) |
| - | Laemmli SDS sample buffer | 65mM Tris-HCl, 10% glycerol, 2% SDS, 0.025% bromophenol blue |
| 28390 | MES-buffered saline pH 4.7 | 0.1M MES, 150mM NaCl pH 4.7 |
| 28374 | Modified Dulbecco's PBS | 8mM sodium phosphate, 2mM potassium phosphate, 0.14M NaCl, 10mM KCl, pH 7.4 |
| 28382 | Na carb-bicarb pH 9.4 | 0.2M sodium carbonate-bicarbonate pH 9.4 |
| 28388 | Na citrate-carbonate pH 9 | 0.6M sodium citrate, 0.1M sodium-carbonate pH 9 |
| 28386 | Na citrate-MOPS pH 7.5 | 0.6M sodium citrate 0.1M MOPS pH 7.5 |
| 28372 | Phosphate-buffered saline (PBS) | 100mM sodium phosphate, 150mM NaCl pH 7.2 |
| 89900 | RIPA Buffer | 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP40, 0.1% SDS pH 8.0 |
| 28379 | Tris-buffered saline (TBS) | 25mM Tris, 150mM NaCl pH 7.6 |
| 28380 | Tris-glycine pH 8.0 | 25mM Tris, 192mM glycine pH 8.0 |
| 28378 | Tris-glycine-SDS pH 8.3 | 25mM Tris, 192mM glycine, 0.1% SDS pH 8.3 |
| 28398 | Tris-HEPES-SDS | 100mM Tris, 100mM HEPES, 3mM SDS |

Thermo Scientific Pierce Protein Assay

| Test Compound | 660 nm | BCA | Micro BCA | ††Microplate BCA-RAC | Coomassie Plus | Coomassie | Modified Lowry |
|----------------------------------|--------|--------|-----------|-------------------------|-------------------|-----------|-------------------|
| 2-D sample buffer ‡ | neet | n/a | n/a | n/a | n/a | n/a | n/a |
| 2-Mercaptoethanol | 1M | 0.01% | 1mM | 25mM (35) | 1M | 1M | 1mM |
| ACES, pH 7.8 | 50mM | 25mM | 10mM | ∅ | 100mM | 100mM | n/a |
| Acetone | 50% | 10% | 1% | ∅ | 10% | 10% | 10% |
| Acetonitrile | 50% | 10% | 1% | 30% | 10% | 10% | 10% |
| Ammonium sulfate | 125mM | 1.5M | ∅ | ∅ | 1M | 1M | ∅ |
| Aprotinin | 2mM | 10mg/L | 1mg/L | ∅ | 10mg/L | 10mg/L | 10mg/L |
| Ascorbic acid | 500mM | ∅ | ∅ | n/a | 50mM | 50mM | 1mM |
| Asparagine | 40mM | 1mM | n/a | ∅ | 10mM | 10mM | 5mM |
| Bicine | >1M | 20mM | 2mM | 1mM | 100mM | 100mM | n/a |
| Bis-Tris pH 6.5 | 50mM | 33mM | 0.2mM | 16.5mM | 100mM | 100mM | n/a |
| Borate (50mM) pH 8.5 | neet | neet | 1:4 | ∅ | neet | neet | n/a |
| B-PER* Reagent | 1:2 | neet | n/a | 1:3 | 1:2 | 1:2 | n/a |
| B-PER Reagent II | 1:2 | n/a | n/a | 1:4 | 1:4 | n/a | n/a |
| B-PER Reagent PBS | 1:2 | n/a | n/a | 1:4 | n/a | n/a | n/a |
| Brij*-35 | 5% | 5% | 5% | 0.63% | 0.062% | 0.125% | 0.031% |
| Brij-56 | n/a | 1% | 1% | n/a | 0.031% | 0.031% | 0.062% |
| Brij-58 | 5% | 1% | 1% | 0.50% | 0.016% | 0.031% | 0.062% |
| Bromophenol blue (in 50mM NaOH) | 0.031% | ∅ | ∅ | ∅ | ∅ | ∅ | ∅ |
| Calcium chloride (in TBS pH 7.2) | 40mM | 10mM | 10mM | 1mM | 10mM | 10mM | n/a |
| Cesium bicarbonate | 100mM | 100mM | 100mM | ∅ | 100mM | 100mM | 50mM |
| Cetylpyridinium chloride | 2.5% † | n/a | n/a | n/a | n/a | n/a | n/a |
| CHAPS | 5% | 5% | 1% | 10% (10) | 5% | 5% | 0.062% |
| CHAPSO | 4% | 5% | 5% | ∅ | 5% | 5% | 0.031% |
| CHES | >500mM | 100mM | 100mM | 50mM | 100mM | 100mM | n/a |
| Cobalt chloride (in TBS pH 7.2) | 20mM | 0.8mM | ∅ | 0.4mM | 10mM | 10mM | n/a |
| CTAB | 2.5% † | n/a | n/a | n/a | n/a | n/a | n/a |
| Cysteine | 350mM | ∅ | ∅ | 2.5mM | 10mM | 10mM | 1mM |
| Dithioerythritol (DTE) | 25mM | 1mM | ∅ | 2.5mM | 1mM | 1mM | ∅ |
| Dithiothreitol (DTT) | 500mM | 1mM | ∅ | 5mM (5) | 5mM | 5mM | ∅ |
| DMF | 50% | 10% | 1% | 5% | 10% | 10% | 10% |
| DMSO | 50% | 10% | 1% | 0.25% | 10% | 10% | 10% |
| DTAB | 2% † | n/a | n/a | n/a | n/a | n/a | n/a |
| EDTA | 20mM | 10mM | 0.5mM | 5mM (20) | 100mM | 100mM | 1mM |
| EGTA | 20mM | ∅ | ∅ | 5mM (10) | 2mM | 2mM | 1mM |
| EPPS pH 8.0 | 200mM | 100mM | 100mM | ∅ | 100mM | 100mM | n/a |
| Ethanol | 50% | 10% | 1% | ∅ | 10% | 10% | 10% |
| Ferric chloride (in TBS pH 7.2) | 5mM | 10mM | 0.5mM | 5mM | 10mM | 10mM | n/a |
| Glucose | 500mM | 10mM | 1mM | ∅ | 1M | 1M | 100mM |
| Glutathione (reduced) | 100mM | n/a | n/a | 10mM | n/a | n/a | n/a |
| Glycerol (fresh) | 50% | 10% | 1% | 5% | 10% | 10% | 10% |
| Glycine-HCl pH 2.8 | 100mM | 100mM | n/a | 50mM | 100mM | 100mM | 100mM |
| Guanidine-HCl | 2.5M | 4M | 4M | 1.5M (2) | 3.5M | 3.5M | n/a |
| HEPES pH 7.5 | 100mM | 100mM | 100mM | 200mM (200) | 100mM | 100mM | 1mM |
| Hydrides (Na2BH4 or NaCNBH3) | ∅ | ∅ | ∅ | n/a | n/a | n/a | n/a |
| Hydrochloric acid (HCl) | 125mM | 100mM | 10mM | ∅ | 100mM | 100mM | 100mM |
| Imidazole pH 7.0 | 200mM | 50mM | 12.5mM | 30mM (50) | 200mM | 200mM | 25mM |
| I-PER* Reagent | 1:4 | neet | n/a | n/a | n/a | n/a | n/a |
| Laemmli SDS sample buffer ‡ | neet † | ∅ | ∅ | ∅ | ∅ | ∅ | ∅ |
| Leupeptin | 80µM | 10mg/L | 10mg/L | ∅ | 10mg/L | 10mg/L | 10mg/L |
| Mannitol | 100mM | n/a | n/a | n/a | n/a | n/a | n/a |
| Melibiose | 500mM | ∅ | n/a | n/a | 100mM | 100mM | 25mM |
| Mem-PER* Reagent | neet | neet | neet | 1:2 | neet | n/a | n/a |
| MES-buffered saline pH 4.7 ‡ | neet | neet | 1:4 | ∅ | neet | neet | n/a |
| MES pH 6.1 | 125mM | 100mM | 100mM | 100mM (100) | 100mM | 100mM | 125mM |
| Methanol | 50% | 10% | 1% | 0.5% | 10% | 10% | 10% |
| Magnesium chloride | >1M | n/a | n/a | 100mM | n/a | n/a | n/a |
| Modified Dulbecco's PBS ‡ | neet | neet | neet | neet | neet | neet | n/a |
| MOPS pH 7.2 | 125mM | 100mM | 100mM | 200mM | 100mM | 100mM | n/a |
| M-PER* Reagent | 1:2 | neet | n/a | 1:2 | neet | n/a | n/a |

Continued...

Thermo Scientific Pierce Protein Assay

| Test Compound | 660 nm | BCA | Micro BCA | ††Microplate BCA-RAC | Coomassie Plus | Coomassie | Modified Lowry |
|-----------------------------------|-------------|----------|-----------|-------------------------|-------------------|-----------|-------------------|
| n-Acetylglucosamine | 100mM | 10mM | ∅ | ∅ | 100mM | 100mM | n/a |
| Na acetate pH 4.8 | 100mM | 200mM | 200mM | ∅ | 180mM | 180mM | 200mM |
| Na azide | 0.125% | 0.2% | 0.20% | 0.01% | 0.5% | 0.5% | 0.2% |
| Na bicarbonate | 100mM | 100mM | 100mM | ∅ | 100mM | 100mM | 100mM |
| Na carb-bicarbonate pH 9.4 ‡ | 1:3 | neet | neet | neet | neet | neet | n/a |
| Na chloride | 1.25M | 1M | 1M | 150mM | 5M | 5M | 1M |
| Na citrate pH 4.8 | 12.5mM | 200mM | 5mM | 50mM | 200mM | 200mM | n/a |
| Na citrate-carbonate pH 9 ‡ | ∅ | 1:8 | 1:600 | ∅ | neet | neet | n/a |
| Na citrate-MOPS pH 7.5 ‡ | 1:16 | 1:8 | 1:600 | neet | n/a | neet | n/a |
| Na deoxycholate (DOC) | 0.25% | 5% | 5% | n/a | 0.4% | 0.05% | n/a |
| Na hydroxide (NaOH) | 125mM | 100mM | 50mM | ∅ | 100mM | 100mM | 100mM |
| Na phosphate | 500mM | 100mM | 100mM | 100mM | 100mM | 100mM | 100mM |
| NE-PER* Reagent (CER) | neet | neet | n/a | 1:2 | 1:4 | n/a | n/a |
| NE-PER Reagent (NER) | neet | neet | n/a | 1:4 | neet | n/a | n/a |
| Nickel chloride (in TBS pH 7.2) | 10mM | 10mM | 0.2mM | ∅ | 10mM | 10mM | n/a |
| NP-40 | 5% | 5% | 5% | ∅ | 0.5% | 0.5% | 0.016% |
| Octyl beta-glucoside | 5% | 5% | 0.1% | 2.5% (10) | 0.5% | 0.5% | 0.031% |
| Octylthioglucoside | 10% | 5% | 5% | 7% | 3% | 3% | n/a |
| Na-orthovanadate (in PBS pH 7.2) | 50mM | 1mM | 1mM | 0.5mM | 1mM | 1mM | n/a |
| Phenol Red | 0.5mg/mL | ∅ | ∅ | 3.125µg/mL | 0.5mg/mL | 0.5mg/mL | n/a |
| Phosphate-buffered saline (PBS) ‡ | neet | neet | neet | neet | neet | neet | n/a |
| PIPES pH 6.8 | 100mM | 100mM | 100mM | 25mM | 100mM | 100mM | n/a |
| PMSF in isopropanol | 1mM | 1mM | 1mM | 0.125mM | 1mM | 1mM | 1mM |
| Potassium thiocyanate | 250mM | 3M | n/a | ∅ | 3M | 3M | 100mM |
| P-PER Reagent | 1:2 | ∅ | n/a | 1:2 | ∅ | ∅ | ∅ |
| RIPA buffer ‡ | neet | neet | 1:10 | 1:2 | 1:40 | 1:10 | n/a |
| SDS | 0.01%, 5% † | 5% | 5% | 5% (10) | 0.016% | 0.125% | 1% |
| Sodium compounds | (see Na) | (see Na) | (see Na) | (see Na) | (see Na) | (see Na) | (see Na) |
| Span** 20 | n/a | 1% | 1% | n/a | 0.5% | 0.5% | 0.25% |
| Sucrose | 50% | 40% | 4% | 40% (40) | 10% | 10% | 7.5% |
| TCEP | 40mM | ∅ | ∅ | 10mM (10) | 125mM | 125mM | n/a |
| Thimerosal | 0.25% | 0.01% | ∅ | 0.03% | 0.01% | 0.01% | 0.01% |
| Thiourea | 2M | n/a | n/a | n/a | n/a | n/a | n/a |
| TLCK | 5mg/mL | 0.1mg/L | 0.1mg/L | ∅ | 0.1mg/mL | 0.1mg/L | 0.01mg/L |
| TPCK | 4mg/mL | 0.1mg/L | 0.1mg/L | ∅ | 0.1mg/mL | 0.1mg/L | 0.1mg/L |
| T-PER* Reagent | 1:2 | 1:2 | n/a | n/a | neet | n/a | n/a |
| Tricine pH 8.0 | 500mM | 25mM | 2.5mM | 0.5mM | 100mM | 100mM | n/a |
| Triethanolamine pH 7.8 | 100mM | 25mM | 0.5mM | 25mM | 100mM | 100mM | n/a |
| Tris-buffered saline (TBS) ‡ | neet | neet | 1:10 | neet | neet | neet | n/a |
| Tris-glycine pH 8.0 ‡ | neet | 1:3 | 1:10 | ∅ | neet | neet | n/a |
| Tris-glycine-SDS pH 8.3 ‡ | neet † | neet | neet | ∅ | 1:4 | 1:2 | n/a |
| Tris-HCl pH 8.0 | 250mM | 250mM | 50mM | 35mM (50) | 2M | 2M | 10mM |
| Tris-HEPES-SDS ‡ | neet † | n/a | n/a | n/a | n/a | n/a | n/a |
| Triton** X-100 | 1% | 5% | 5% | 7% (10) | 0.062% | 0.125% | 0.031% |
| Triton X-114 | 0.50% | 1% | 0.05% | 2% (2) | 0.062% | 0.125% | 0.031% |
| Triton X-305 | 9% | 1% | 1% | 1% | 0.125% | 0.5% | 0.031% |
| Triton X-405 | 5% | 1% | 1% | ∅ | 0.025% | 0.5% | 0.031% |
| Tween** 20 | 10% | 5% | 5% | 10% (10) | 0.031% | 0.062% | 0.062% |
| Tween 60 | 5% | 5% | 0.5% | 5% | 0.025% | 0.1% | n/a |
| Tween 80 | 5% | 5% | 5% | 2.5% | 0.016% | 0.062% | 0.031% |
| Urea | 8M | 3M | 3M | 3M (4) | 3M | 3M | 3M |
| Y-PER* Reagent | ∅ | neet | n/a | n/a | n/a | n/a | n/a |
| Y-PER Plus Reagent | 1:2 | neet | n/a | n/a | neet | n/a | n/a |
| Zinc chloride (in TBS pH 7.2) | 10mM | 10mM | 0.5mM | ∅ | 10mM | 10mM | n/a |
| Zwittergent** 3-14 | 0.05% | 1% | ∅ | 2% (2) | 0.025% | 0.025% | n/a |

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