Histone Purification Mini Kit

(version B3)

Catalog No. 40026

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Introduction

Active Motif's Histone Purification Mini Kit enables you to purify the core histone proteins and enrich for histone fractions while preserving their post-translational modifications (e.g. acetylation, methylation, and phosphorylation). This purification method is an improvement over acid precipitation methods and utilizes a convenient spin column and a proprietary buffer system to purify the core histones from cells and tissue samples. Histones isolated by this method are suitable substrates for downstream assays and *in vitro* chromatin assembly.

Unlike standard acid extraction techniques, this kit uses proprietary technology to purify the core histones. Post-translational modifications such as phosphorylation, acetylation and methylation are preserved, so you can extract core histone proteins from your cell culture or animal model and determine which modifications are present.

The Histone Purification Mini Kit provides reagents for 20 histone purifications from as little as $8x10^5$ cells up to grams of tissue due to the robust histone binding capacity of the purification column. The kit method is simple: first, an extract is made and applied to the purification column, then histones are eluted, enabling the purification of core histones. Histones may be quantified by an OD reading or quantitated empirically on a gel by comparison with histone standards.

product	format	catalog no.
Histone Purification Mini Kit	20 rxns	40026

The Histone Purification Mini Kit is for research use only. Not for use in diagnostic procedures.

Kit Performance

Core Histones Purified from Cells and Tissue

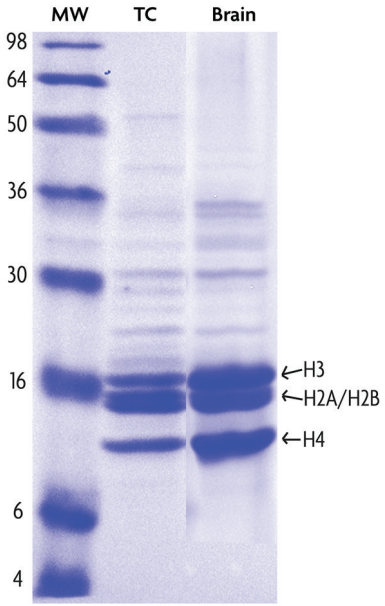
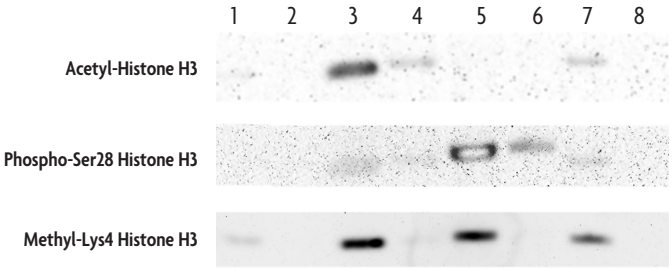


Figure 1: SDS-PAGE of histone fractions purified using the Histone Purification Mini Kit.

Ten µg per lane of core histones purified from logarithmically growing tissue culture cells (TC) and core histones isolated from rat brain tissue (Brain) were loaded and run on a 16% Tris-glycine gel.

Histone Purification Mini Kit preserves post-translational modifications

Active Motif's Histone Purification Mini Kit preserves phosphoryl, acetyl and methyl post-translational modifications on histones. The Western blot data shown below of the first and second elutions of unstimulated HeLa (lanes 1-2), sodium butyrate-treated HeLa (lanes 3-4), paclitaxel-treated HeLa (lanes 5-6) and rat brain histones (lanes 7-8) demonstrates that post-translational modifications are intact following purification of histones.



Yield of Histones:

The following yields are approximate. Results may vary according to cell or tissue type. It is possible to obtain a decent yield (10 µg) of histones from as few as 8 x 10^5 mammalian cells.

Adherent Cells: 0.1 mg total core histones from 8 x 10^6 cells (one 150 mm plate).

Suspension Cells: 0.1 mg total core histones from 8 x 10^6 cells.

Tissue: 1 mg histone per gram of tissue*

* The theoretical binding limit for these columns is 1 mg, but this has not been confirmed empirically. Loading more than the theoretical limit is not recommended.

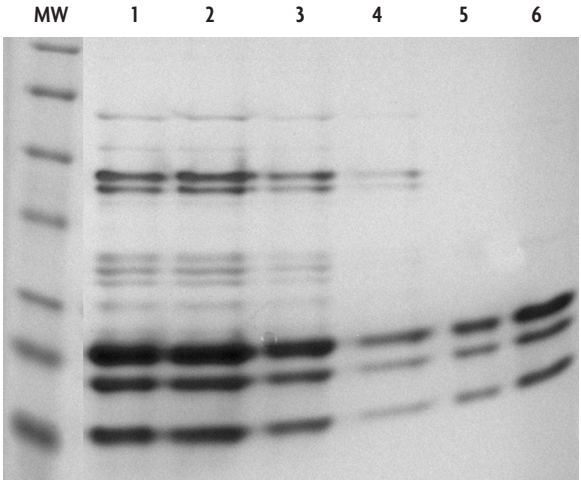


Figure 2: Analysis of histone yield using varying numbers of HeLa cells.

Histones were extracted from different numbers of HeLa cells using the Histone Purification Mini Kit and loaded onto a 16% SDS-PAGE gel. Purified HeLa core histones were loaded as a standard in parallel. On this particular gel system, H2A and H2B exhibit coincident migration.

Lane 1: 15 µl of a 100 µl elution from histones purified from a single 150 mm tissue culture plate of HeLa cells at 100% confluency (8 x 10^6 cells).

Lane 2: 15 µl of a 50 µl elution from histones purified from half of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (4 x 10^6 cells).

Lane 3: 15 µl of a 50 µl elution from histones purified from one-fifth of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (1.6 x 10^6 cells).

Lane 4: 15 µl of a 50 µl elution from histones purified from one-tenth of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (8 x 10^5 cells).

Lane 5: 2.5 µg of purified HeLa core histones.

Lane 6: 5 µg of purified HeLa core histones.

Kit Components and Storage

Please store each component at the temperature indicated below. All components are guaranteed stable for 6 months from date of purchase when stored at the appropriate temperatures.

Reagents	Quantity	Storage / Stability
5X Neutralization Buffer	50 ml	4°C
Extraction Buffer	100 ml	4°C
Equilibration Buffer	10 ml	4°C
Histone Wash Buffer	30 ml	4°C
Histone Elution Buffer	10 ml	4°C
Purification Spin Columns	20	Room Temp
Collection Tubes	40	Room Temp

Additional materials required

- Dounce homogenizer with a small clearance pestle (e.g. Active Motif cat. nos. 40401 or 40415; the large pestles supplied with these Dounces can be used for initial sample reduction, while the small pestles should be used to process the final homogenate).
- 1.7 ml microcentrifuge tubes
- Sterile water or Tris-EDTA, pH 8.0
- SDS sample buffer
- SDS-PAGE and Western blot reagents
- For precipitation of histones: Perchloric acid, 70% (Acros Organics, part no. 424030010)
- For washing of histones:
  4% perchloric acid
  0.2% HCl in acetone*
  Cold 100% acetone (VWR, part no. BDH1101)

* Add 0.5 ml of HCl stock (36.8%, Sigma, part no. H-7020) and adjust the volume with acetone until 92 ml. Store in a glass bottle at 4°C.

Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Step 1: Prepare Sample Extracts

For adherent cells:

1. Grow cells to 80-90% confluence in the appropriate medium. Discard media and wash the cells 2 times with pre-warmed (37°C) serum-free media: use 2 ml for a 35 mm dish; 10 ml for a 100 mm dish; or 20 ml for a 150 mm dish.
2. After the 2nd wash, aspirate any remaining wash media and add ice-cold Extraction Buffer to each dish: use 0.3 ml for each 35 mm dish; 0.8 ml for a 100 mm dish; or 1.5 ml for a 150 mm dish. Using a plastic scraper and a pipet, collect the cell protein extracts in a 1.7 ml microcentrifuge tube or a 15 ml tube as dictated by the amount of material. Pipet the cells up and down to homogenize them into the solution.

For suspension cells:

1. Grow cells to about 80-90% confluence in appropriate medium. If necessary, gently scrape the cells from the sides of the dish while keeping them in their media.
2. Pipet the cells with the media and transfer to a 15 ml tube.
3. Centrifuge at 1,000 x g for 5 minutes at room temperature.
4. Wash the cells twice with 20 ml pre-warmed (37°C) serum-free media. After each wash, centrifuge as in No. 3 above, then discard the serum-free media.
5. Resuspend the cells in ice-cold Extraction Buffer (0.5 ml per 150 cm^2 flask yields good concentrated extracts). Pipet up and down to homogenize the cells well, then transfer the resuspended cells to a microcentrifuge tube.

For tissues:

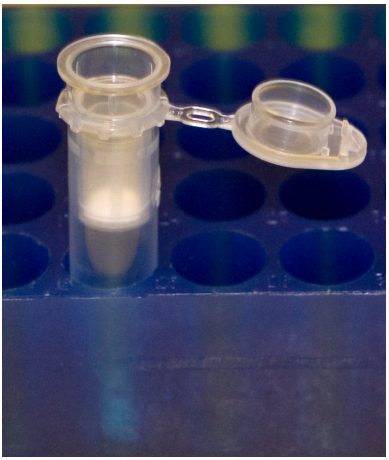
1. Homogenize the tissue completely in an ice-cold Dounce homogenizer using as little ice-cold Extraction Buffer as possible, as this will help ensure a highly concentrated extract. Keep the homogenate on ice.
2. Transfer the homogenate into a fresh microcentrifuge tube. Continue with Step 2: Prepare Crude Core Histone Extract on page 6.

Step 2: Prepare Crude Core Histone Extract

1. Leave the cells in Extraction Buffer for 30 minutes to 2 hours (or overnight) on a rotating platform at 4°C. (Time course experiments performed on some cell lines have shown that most histones are extracted in as little as 10 minutes).
2. Transfer the cell extracts to fresh tubes and centrifuge at maximum speed in a microfuge for 5 minutes at 4°C.
3. Transfer the supernatant, which contains the crude histones, to a new tube. The pellet can be discarded or stored at -20°C for future analysis. If the pellet is to be stored, neutralize it by adding 100 µl of 1 M Tris HCl pH 8.0.
4. Store the crude histones at -80°C, or continue with the next step.
5. Neutralize and equilibrate the crude histones with 1/4 volume of 5X Neutralization Buffer. For example, add 0.25 ml of 5X Neutralization Buffer to 1 ml crude histones, 0.5 ml (5X) to 2 ml, 1 ml (5X) to 4 ml of crude histones, etc.
6. Check the pH. If still acidic, add additional 5X Neutralization Buffer until the pH reaches 8.
7. It is recommended to remove an aliquot of the crude histones for comparison to the purified histones (column input control).

Step 3: Purify Core Histones

1. Equilibrate the spin columns by adding 0.5 ml Equilibration Buffer then centrifuging at 2,000 x g (approximately 4,500 rpm) for 5 minutes at 4°C.



2. Add 0.5 ml of the sample from Step 2 to the column and centrifuge at 2,000 x g for 5 minutes at 4°C. Remove the flow-through and save to a new 1.7 ml microcentrifuge tube. Repeat this step as many times as necessary to load the entire sample on the column. (The flow-throughs can be combined and analyzed to determine column binding efficiency.)

3. Wash the column with 0.5 ml Histone Wash Buffer and centrifuge at 2,000 x g for 5 minutes at 4°C. Remove the wash buffer to a microfuge tube and save for analysis later. Repeat this step twice for a total of three washes.
4. Transfer the column to a new 2 ml round-bottom microcentrifuge tube. Add 100 µl of Histone Elution Buffer and centrifuge at 2,000 x g for 5 minutes at 4°C. Save the flow-through containing the eluted histone proteins.
5. **Optional second elution:** repeat step 4, if desired, keeping the 2nd eluate separate from the first eluate.

At this point the proteins are ready for use, but contain high levels of salts in the buffer that may interfere with some downstream binding assays or other applications. If desired, the proteins can be used without precipitation after being desalted using columns designed for this purpose (e.g. Zeba spin columns, Thermo Fisher part no. 89889). To further concentrate the proteins and remove the salt, proceed to Step 4: Precipitate Histone Proteins.

Step 4: Precipitate Histone Proteins

1. Precipitate the histone proteins overnight at 4°C by adding perchloric acid to a final concentration of 4%. For example add 6 µl of 70% perchloric acid to 100 µl fraction, then vortex.
2. On the following day, spin the samples at maximum speed in a microfuge for 1 hour at 4°C.
3. Gently wash the histone pellet with 1 ml cold 4% perchloric acid. Do not vortex the pellet. Centrifuge for 5 minutes at maximum speed in a microfuge. Repeat this step for a total of two washes.
4. Wash the histone pellet two times as in step 3 using ice cold acetone containing 0.2% HCl.
5. Wash the histone pellet two times as in step 3 using ice cold acetone.
6. Air dry the pellet until it is completely dry, around 10 to 20 minutes.
7. Resuspend in sterile water or TE. Flick the bottom of the tube gently with a finger. Let the pellet resuspend 10 to 20 minutes at room temperature, then gently vortex and centrifuge briefly. Check to make sure that pellet is completely resuspended. Store at -20°C or -80°C.

Step 5: Quantify Yield of Histone Proteins

1. Total core histone proteins can be quantified by measuring the absorbance at 230 nm. An OD of 0.42 from a sample diluted 1:10 indicates a protein concentration of 1 mg/ml.

   Undiluted samples may be outside the linear range of your spectrophotometer. Therefore, we recommend preparing a 1/10 dilution of your samples before quantifying.

$$\text{OD of } 0.42 = 1 \text{ mg / ml}$$

$$\frac{\text{OD } 0.42}{1 \text{ mg/ml}} = \frac{\text{Your OD}}{x \text{ mg / ml}}$$

Solve for x = Concentration of diluted stock
Multiply by 10 = Actual histone concentration

Appendix

Section A: Troubleshooting Guide

Problem/question	Recommendation
Clogging problems appear when loading the sample onto the column.	Perform an additional 5 minute centrifugation step at 2,000 x g.
In the precipitation step, there is no visible pellet after spinning the histone samples.	In general, a white pellet is visible. Leave some of washing solutions to avoid disturbing the pellet; do not vortex it. Also, the histones will not precipitate well if the preparation is too dilute. Elute the sample in less volume to obtain more concentrated preparations.
Is it possible to eliminate H1 from the final preparation of histones?	Yes. Precipitate the histone proteins in perchloric acid 4% (H1 protein remains soluble). H1 protein can be recovered by re-precipitation of the soluble fraction using 20-30% trichloroacetic acid.
Do the histone proteins react with Coomassie dye while in solution?	No. Core histones react poorly with Coomassie dye while in solution, and H1 does not react at all. However, H1 and core histones are stained by Coomassie effectively in gel.

Section B: Related Products

Histone Antibodies

For an up-to-date list of over 220 antibodies against histones and modified histones, please visit **www.activemotif.com/histoneabs**.

ChIP-validated Antibodies

For an up-to-date list of over 250 ChIP-validated antibodies, please visit **www.activemotif.com/chipabs**.

Chromatin Immunoprecipitation	Format	Catalog No.
ChIP-IT® Express	25 rxns	53008
ChIP-IT® Express Enzymatic	25 rxns	53009
ChIP-IT® Express Shearing Kit	10 rxns	53032
ChIP-IT® Express Enzymatic Shearing Kit	10 rxns	53035
ChIP-IT® Express HT	96 rxns	53018
Re-ChIP-IT®	25 rxns	53016
RNA ChIP-IT®	25 rxns	53024
Chromatin IP DNA Purification Kit	50 rxns	58002
EpiShear™ Sonicator	110 V	53051
ChIP-IT® Control Kit – Human	5 rxns	53010
ChIP-IT® Control Kit – Mouse	5 rxns	53011
ChIP-IT® Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
Bridging Antibody for Mouse IgG	500 µg	53017
ChIP-IT® Protein G Magnetic Beads	25 rxns	53014
Siliconized Tubes, 1.7 ml	25 tubes	53036

Co-Immunoprecipitation	Format	Catalog No.
Universal Magnetic Co-IP Kit	25 rxns	54002
Nuclear Complex Co-IP Kit	50 rxns	54001

Modified Histones Array	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001

Histone Modification FP Binding Assay	Format	Catalog No.
HiLite™ Histone H3 Methyl-Lys9 / Lys27 FP Binding Assay	1 kit	57001

Histone Purification & Chromatin Assembly	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026
Histone Purification Microplate Kit	96 rxns	40027
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Histone ELISAs	Format	Catalog No.
Total Histone H3 ELISA	1 x 96 rxns	53110
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 acetyl Lys9 ELISA	1 x 96 rxns	53114
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 acetyl Lys14 ELISA	1 x 96 rxns	53115
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100

Recombinant Methylated, Acetylated & Phosphorylated Histone Proteins	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	100 µg	31207
Recombinant Histone H3 biotinylated	25 µg	31271
Recombinant Histone H3 phospho Thr3 (H3T3ph)	25 µg	31274
Recombinant Histone H3 monomethyl Lys4 (H3K4me1)	50 µg	31208
Recombinant Histone H3 dimethyl Lys4 (H3K4me2)	50 µg	31209
Recombinant Histone H3 trimethyl Lys4 (H3K4me3)	50 µg	31210

Recombinant Histone Proteins (continued)	Format	Catalog No.

Recombinant Histone Proteins (continued)	Format	Catalog No.
Recombinant Histone H3 acetyl Lys9 (H3K9ac)	25 µg	31253
Recombinant Histone H3 monomethyl Lys9 (H3K9me1)	50 µg	31211
Recombinant Histone H3 dimethyl Lys9 (H3K9me2)	50 µg	31212
Recombinant Histone H3 trimethyl Lys9 (H3K9me3)	50 µg	31213
Recombinant Histone H3 phospho Ser10 (H3S10ph)	25 µg	31272
Recombinant Histone H3 acetyl Lys14 (H3K14ac)	25 µg	31254
Recombinant Histone H3 monomethyl Lys14 (H3K14me1)	50 µg	31256
Recombinant Histone H3 dimethyl Lys14 (H3K14me2)	50 µg	31257
Recombinant Histone H3 trimethyl Lys14 (H3K14me3)	50 µg	31258
Recombinant Histone H3 acetyl Lys18 (H3K18ac)	25 µg	31273
Recombinant Histone H3 monomethyl Lys18 (H3K18me1)	50 µg	31259
Recombinant Histone H3 dimethyl Lys18 (H3K18me2)	50 µg	31260
Recombinant Histone H3 trimethyl Lys18 (H3K18me3)	50 µg	31261
Recombinant Histone H3 acetyl Lys23 (H3K23ac)	25 µg	31255
Recombinant Histone H3 monomethyl Lys23 (H3K23me1)	50 µg	31262
Recombinant Histone H3 dimethyl Lys23 (H3K23me2)	50 µg	31263
Recombinant Histone H3 trimethyl Lys23 (H3K23me3)	50 µg	31264
Recombinant Histone H3 monomethyl Lys27 (H3K27me1)	50 µg	31214
Recombinant Histone H3 dimethyl Lys27 (H3K27me2)	50 µg	31215
Recombinant Histone H3 trimethyl Lys27 (H3K27me3)	50 µg	31216
Recombinant Histone H3 monomethyl Lys36 (H3K36me1)	50 µg	31217
Recombinant Histone H3 dimethyl Lys36 (H3K36me2)	50 µg	31218
Recombinant Histone H3 trimethyl Lys36 (H3K36me3)	50 µg	31219
Recombinant Histone H3 monomethyl Lys79 (H3K79me1)	50 µg	31220
Recombinant Histone H3 dimethyl Lys79 (H3K79me2)	50 µg	31221
Recombinant Histone H3 trimethyl Lys79 (H3K79me3)	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys5 (H4K5me1)	50 µg	31265
Recombinant Histone H4 dimethyl Lys5 (H4K5me2)	50 µg	31266
Recombinant Histone H4 trimethyl Lys5 (H4K5me3)	50 µg	31267
Recombinant Histone H4 monomethyl Lys16 (H4K16me1)	50 µg	31268
Recombinant Histone H4 dimethyl Lys16 (H4K16me2)	50 µg	31269
Recombinant Histone H4 trimethyl Lys16 (H4K16me3)	50 µg	31270
Recombinant Histone H4 monomethyl Lys20 (H4K20me1)	50 µg	31224
Recombinant Histone H4 dimethyl Lys20 (H4K20me2)	50 µg	31225
Recombinant Histone H4 trimethyl Lys20 (H4K20me3)	50 µg	31226

For an up-to-date list of Recombinant Histone Proteins, please visit **www.activemotif.com/recombhis**.

Histone Demethylase Activity	Format	Catalog No.
Histone Demethylase Assay (Fluorescent)	48 rxns	53200

DNA Methylation	Format	Catalog No.
hMeDIP	10 rxns	55010
MeDIP	10 rxns	55009
MethylDetector™	50 rxns	55001
MethylCollector™ Ultra	30 rxns	55005
DNMT Activity / Inhibition Assay	96 rxns	55006
Methylated DNA Standard Kit	3 x 2.5 µg	55008
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

DNA Methylation Antibodies

For an up-to-date list of over 25 DNA Methylation antibodies, visit **www.activemotif.com/dnamethabs**.

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

In-cell Phospho-specific ELISAs	Format	Catalog No.	Catalog No.
FACE™ AKT	1 x 96 rxns	48120	48220
FACE™ ATF-2	1 x 96 rxns	48115	48215
FACE™ Bad	1 x 96 rxns	48165	48265
FACE™ c-Jun (S63)	1 x 96 rxns	48125	48225
FACE™ c-Jun (S73)	1 x 96 rxns	48135	48235
FACE™ c-Src	1 x 96 rxns	48155	48255
FACE™ EGFR (Y845)	1 x 96 rxns	48340	48440
FACE™ EGFR (Y992)	1 x 96 rxns	48150	48250
FACE™ EGFR (Y1173)	1 x 96 rxns	48190	48290
FACE™ ErbB-2 (Y877)	1 x 96 rxns	48130	48230
FACE™ ErbB-2 (Y1248)	1 x 96 rxns	48105	48205
FACE™ ERK1/2	1 x 96 rxns	48140	48240
FACE™ FAK	1 x 96 rxns	48145	48245
FACE™ FKHR (FOXO1)	1 x 96 rxns	48160	48260
FACE™ HSP27	1 x 96 rxns	48350	48450
FACE™ JAK1	1 x 96 rxns	48185	48285
FACE™ JNK	1 x 96 rxns	48110	48210
FACE™ MEK1/2	1 x 96 rxns	48180	48280
FACE™ NFκB p65 Profiler	3 x 96 rxns	48300	48400
FACE™ p38	1 x 96 rxns	48100	48200
FACE™ PI3 Kinase p85	1 x 96 rxns	48175	48275
FACE™ STAT2	1 x 96 rxns	48310	48410
FACE™ STAT4	1 x 96 rxns	48320	48420
FACE™ STAT6	1 x 96 rxns	48330	48430
FACE™ Maker	1 x 96 rxns	48000	48050
Suspension Cell FACE™	2 x 96 rxns	48305	48405

For a complete, up-to-date list of available FACE™ Kits, please visit **www.activemotif.com/face**

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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