SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P312-37C-25	25 µg
P312-37C-100	100 µg

PAD Cocktail, Active

Recombinant protein expressed in Sf9 cells

Catalog # P312-37C
Lot # Q2535-2

Product Description

The five full length recombined PAD proteins (1, 2, 3, 4 and 6) expressed by baculovirus in Sf9 insect cells using N-terminal GST tags. This mixture is ideally suited for in vitro citrullination of protein targets.

Molecular Weight

PAD1: approx. 95 kDa
PAD2: approx. 99 kDa
PAD3: approx. 97 kDa
PAD4: approx. 96 kDa
PAD6: approx. 100 kDa

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

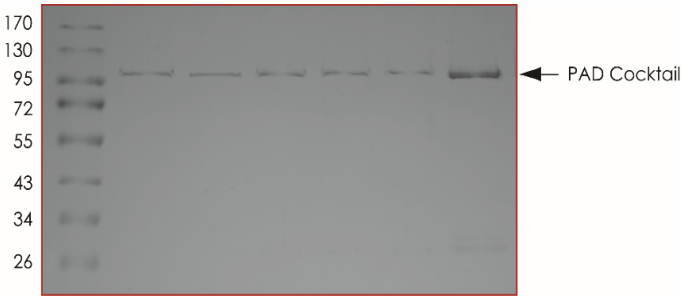
Store product at –70°C. For optimal storage, aliquot cocktail into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Specific Activity

Sample Mass Spectrometry Data

Peptide Sequence	PAD Treated	Negative Control
XXRXXXXXXXXX	5 reads, 3 citrullination	No citrullination
XXXXXXXXXXRXXXRXXXX	8 reads, 4 citrullination	No citrullination
XXXXXXRXXXXXXXX	16 reads, 6 citrullination	No citrullination

Purity



SDS-PAGE image of PAD Cocktail Proteins. The five PAD isoform proteins from left to right are: PAD1, PAD4, PAD3, PAD6 and PAD2. The far right lane contains PAD Cocktail. The purity of PAD cocktail was determined to be **>95%** by densitometry, approx. MW **95~100 kDa**.

PAD Cocktail, Active

Recombinant protein expressed in Sf9 cells

Catalog #	P312-37C
Lot #	Q2535-2
Purity	>95%
Concentration	0.5 µg/µl (Each PAD at 0.1 µg/µl)
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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www.signalchem.com

In Vitro Citrullination Protocol

Reaction Components

PAD Cocktail, Active (Catalog #: P312-37C)

10µl active PAD Cocktail (5µg of total PAD cocktail proteins) diluted with 20µl of PAD Cocktail Dilution Buffer (0.1M Tris-HCl, pH 7.4, 10mM $CaCl_2$) (Note: these are suggested working dilutions and it is recommended that the researcher perform appropriate dilutions for optimal results).

Anti-GST Agarose Beads

Any commercially available or self-made Anti-GST Agarose Beads may be used.

PAD Cocktail Dilution Buffer

Buffer components: 0.1M Tris-HCl, pH 7.4, 0.25mM DTT and 10mM $CaCl_2$.

PAD Buffer

Buffer components: 0.1M Tris-HCl, pH 7.4, 10mM $CaCl_2$ with 5mM DTT, 10µg/µl aprotonin, 10µg/µl leupeptin and 10µg/µl pepstatin.

4x Laemelli Sample Buffer

8% SDS, 40% glycerol, 250mM Tris-HCl (pH 6.8), 0.02% Bromophenol blue, and 8% β-mercaptoethanol.

Substrate Protein

Protein target to be determined by the researcher.

Citrullination Protocol

Step 1. Wash and equilibrate 5µl of anti-GST agarose beads in PAD Cocktail Dilution Buffer. Remove as much buffer as possible after equilibration.

Step 2. Incubate the equilibrated anti-GST agarose beads with 5µg of PAD Cocktail suspended in 20µl of PAD Cocktail Dilution Buffer at 4°C for 1 hour while shaking.

- **Component 1.** 5µl of equilibrated anti-GST agarose beads
- **Component 2.** 10µl of PAD Cocktail, Active (Catalog #P312-37C)
- **Component 3.** 20µl PAD Cocktail Dilution Buffer

Step 3. For a negative control, incubate the equilibrated anti-GST agarose beads in 20µl of PAD Cocktail Dilution Buffer.

Step 4. After incubation, wash the agarose beads three times in PAD buffer.

Step 5. Add 10µg of a Substrate Protein and 100µl PAD buffer to the PAD cocktail conjugated agarose beads and negative control, and incubate at 37°C for 2 hours while shaking.

- **Component 1.** 10µg of Substrate Protein
- **Component 2.** 100µl of PAD Buffer

Step 6. Centrifuge the beads and collect the supernatant containing the citrullinated Substrate Protein.

Step 7. Stop the reaction with 25ul of 4x Laemelli Sample Buffer.

Step 8. Store samples at -80°C until further use.

Step 9. To assess the level of citrullination and characterize the modification, the citrullinated and control samples can be sent for mass spectrometry analysis.

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