

Certificate of Analysis for NR-31212

Mycobacterium bovis, Strain AF 2122/97 (ATCC® BAA-935™), Culture Filtrate Proteins

Catalog No. NR-31212

This reagent is the tangible property of the U.S. Government.

Product Description: NR-31212 is a preparation of culture filtrate proteins (CFP) from *Mycobacterium bovis*, AF 2122/97 (ATCC[®] BAA-935[™]) and contains most of the excreted and secreted proteins of the organism.

Lot^{1,2}: 61056062 Manufacturing Date: 29JAN2012

TEST	SPECIFICATIONS	RESULTS
SDS-PAGE (Silver Stain)	Report results	Multiple protein bands visible (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay³	Report results	1.22 mg per mL
Western Blot Analysis Monoclonal anti-GroES ⁴ Monoclonal anti-Mpt32 ⁵ Monoclonal anti-DnaK ⁶ Monoclonal anti-HspX ⁷	Reactive Reactive Reactive Non-reactive	Reactive (Figure 2) Reactive (Figure 2) Reactive (Figure 2) Non-reactive (Figure 2)

¹Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for bulk lot 12.BAA935.1.3.3.CFP is attached.

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Date: 10 JUN 2017 Signature:

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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NR-14826_61056062_10JUN2017

²Retesting of NR-31212 lot 64056062 was performed by BEI Resources in May 2017 to confirm product stability.

³Using BSA as a standard

⁴M. tuberculosis anti-GroES monoclonal antibody (BEI Resources, NR-49223) (1:100 dilution)

⁵M. tuberculosis anti-Mpt32 monoclonal antibody (BEI Resources, NR-13807) (1:50,000 dilution)

⁶M. tuberculosis anti-DnaK (clone A) monoclonal antibody (BEI Resources, NR-49679) (1:5000 dilution)

⁷M. tuberculosis anti-HspX monoclonal antibody (BEI Resources, NR-13607) (1:00 dilution); Negative control



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Figure 1: SDS-PAGE of NR-31212 Lane 1: Precision Plus Protein™ Standard Lane 2: NR-31212, 24 μg

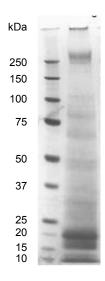
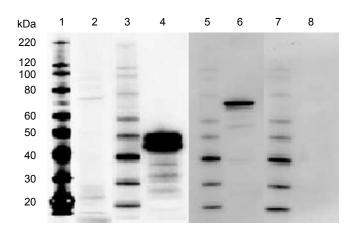


Figure 2: Western Blots of Prominent Culture Filtrate Proteins



Lane 1: MagicMark™ XP Protein Standard Lane 2: NR-31212 (24 µg) with anti-GroES Lane 3: MagicMark™ XP Protein Standard Lane 4: NR-31212 (24 µg) with anti-Mpt32 Lane 5: MagicMark™ XP Protein Standard Lane 6: NR-31212 (24 µg) with anti-DnaK Lane 7: MagicMark™ XP Protein Standard

Lane 8: NR-31212 (24 µg) with anti-HspX; Negative control

WORK SHEET FOR CULTURE FILTRATE PROTEIN

General Information

Lot Number: 12.BAA935.1.3.3.CFP Species: *Mycobacterium bovis*

Strain: BAA935

Date Harvested/Finished: 01/23/12 By: Phillip Knabenbauer

NaN3 Added: Yes or No

Date ultrafiltration started: 01/23/12 Date completed: 01/26/12

Date 10 mM ammonium bicarbonate dialysis started: 01/26/12

date completed: 01/29/12

Quality Control Information

Clarity of product/suspension after dialysis: Clear

Passed through 0.2 µm filter: Yes or No

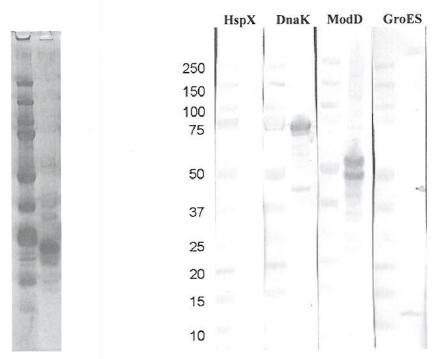
BCA: 1.035 μg /μl. Notebook and page(s): CarolinaQPCR#1 pg.93

Total volume: 200 ml Total amount of Protein: 207 mg
Silver Stain Date: 04/17/12. Notebook and page(s): CarolinaQPCR#1 pg.93

Western Blot of QC Antibodies Date: 04/17/12. Notebook and page(s): __CarolinaQPCR#1 pg.93

QC Gel and Blot:

Hop X is a OCTR; all others me & CTR. &



Aliquot Information:

None

05/01/2012

(date)

(Laboratory Supervisor) 5/2/20/