

BioTechnology Branch CRP Antibody Testing and Repository



The **ECBC Antibody Testing and Repository** provides subject matter expertise, R&D activities, reference material production, bulk testing, and repository services that allow for the enhanced functionality and efficiency of the Critical Reagents Program (CRP) by providing creative solutions for developing high quality detection reagents that are maintained and distributed under the auspices of a validated quality management system.

Antibodies, also known as immunoglobulins (Ig), are recognition proteins produced by plasma cells which are then utilized by the immune system to identify and neutralize pathogens. Their ability to bind to an antigen with such specificity and affinity makes their use in scientific and medical research invaluable. Antibodies are produced and purified in two basic ways: polyclonal and monoclonal. Here, we will highlight just one of the many CRP mAbs (an anti-Vaccinia) that was produced and received for the required testing to be performed for its release.

Monoclonal antibodies (mAbs):

- Identical antibodies
- Target a single epitope
- Produced by fusing antibody-secreting spleen cells from immunized mice with immortal myeloma cells.
- Formed Monoclonal hybridoma cell lines express specific antibody in cell culture supernatant.

BioTechnology's Quality Capability: ISO17025

The CRP Antibody Testing and Repository at ECBC achieved a major landmark with the achievement of **International Standard ISO/IEC 17025:2005 accreditation**. This credential certifies that quality-oriented tests are performed correctly and establishes that the product is in fact a quality product. For a laboratory to be ISO 17025 certified, they must be consistent as well as proficient in testing the quality of their products.

ECBC's scope of accreditation includes; Experion, Dynamic Light Scattering (DLS), Nano-Drop, and Differential Scanning Calorimetry (DSC).



Overview of the Testing Process

All monoclonal antibodies are tested using Dynamic Light Scattering (DLS), Experion, NanoDrop, and Differential Scanning Calorimetry (DSC). For those antibodies that have passed testing, a certificate of analysis (CoA) is generated, approved, and uploaded to the J.A.C.K.S. database, and the antibody is made available for sale. For those antibodies that fail testing, a re-test is performed. If that antibody fails the second test, a non-conformance is issued and the lot is investigated to determine material disposition.

ThermoScientific NanoDrop

The NanoDrop ND-2000 (Thermo Fisher, Madison, WI) Spectrophotometer is used to determine the concentration of the antibody. Each reading requires a 2ul sample which is placed on a sample pedestal and the arm of the instrument is lowered, creating a liquid column- this is the path length through which the laser passed. The instrument is then blanked using PBS w/ 0.1% sodium azide, readings are taken in triplicate.

A positive control, Bovine Gamma Globulin (BGG) (BioRad, Hercules, CA) is also tested with each run to validate the instrument operation. The average of the three replicates is calculated and reported on the Certificate of Analysis (CofA).



	Concentration (mg/mL)
Replicate 1	4.88
Replicate 2	4.94
Replicate 3	5.00
Average	4.90

BioRad Experion Pro260

The Experion Pro260 analysis kit uses engineered lower and upper internal alignment markers to provide clean baselines, accurate molecular weight sizing, and quantitative protein analysis [2]. The Pro260 analytical software also determines sample purity by calculating the percent mass of the separated proteins in a sample. For Experion analysis, antibody is standardized to a final concentration of 1mg/ml by diluting in PBS (Sigma-Aldrich) and creating a 20mL aliquot for all testing. The control (BGG) and sample (Antibody) are then processed using a validated procedure. A Pro260 microfluidic chip is prepared by adding Pro260 gel and gel stain to designated wells. The chip is then placed on the priming station and primed for 1 minute.



Molecular weight and purity data is collected with the Experion (Bio-Rad, Hercules, CA) automated electrophoresis system. It employs microfluidic technology to automate electrophoresis for protein analysis. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software are designed to accomplish separation, staining, detection, and basic data analysis.

The priming fills the fluidic channels with gel, which is used by the instrument to form a barrier between samples during the run. The sample is reduced and denatured with the addition of dithiothrietol (Sigma-Aldrich) and heat before being applied to the primed chip. The chip is then placed in the instrument and the lid closed, lowering the sample needles into the wells. The instrument is operated via the Experion software; each chip takes thirty minutes to complete. All samples are run in triplicate and a compare image is created using one of the three sample gel images and the pro260 ladder (Bio-Rad). All analysis is performed using the Experion software (Bio-Rad). The gel image and percent purity is reported on the certificate of analysis.

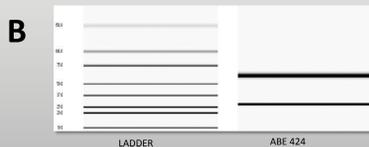


Figure #1. A. The BioRad Experion Pro260, B. Example of gel image included on the CoA.

Wyatt Technologies Dynamic Light Scattering (DLS)

The DLS is used to determine how a protein behaves in solution. DLS data indicates whether a protein is in solution by measuring the polydispersity, hydrodynamic radius, and molecular weight of a sample. Prediction algorithms are employed by the software to produce a range of values for the protein under evaluation. For DLS analysis, five 20ul aliquots of the Antibody and the control Bovine serum albumin (BSA) (Sigma-Aldrich) are placed into a quartz 384 well plate (Wyatt Technology Corporation, Santa Barbara, CA) and centrifuged removing trapped air bubbles from the samples. Paraffin oil is applied to the top of each preventing sample evaporation. The plate is placed into a DynaPro temperature controlled Plate Reader (Wyatt Technology Corporation). Each well is scanned ten times at 25°C, and averaged to provide measurements of polydispersity, hydrodynamic radius, percent mass and molecular weight for each sample using the Wyatt Technology Dynamics software. The results of the five wells are averaged together. Data generated is used internally to qualify the Antibody lot.

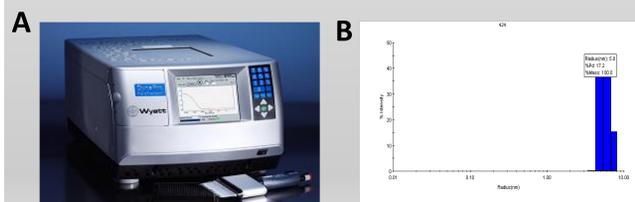


Figure #2. A. The DynaPro Plate Reader B. Example of the regularization graph.

Malvern Differential Scanning Calorimetry (DSC)

DSC is utilized to get a quantitative melting temperature (T_m) of the anti-Vaccinia mAb. The T_m predicts the results of subsequent Enzyme-linked Immunosorbant Assay (ELISA) and Surface Plasmon Resonance (SPR) thermostability testing. A T_m above 70°C predicts that the percent of antibody activity after the thermal stress test should remain above 50%. A T_m below 70°C predicts at least a 50% decrease in antibody activity after the thermal stress test. For DSC experiments, samples are diluted to 0.5mg/ml and dialyzed overnight in PBS (Sigma-Aldrich) pH 7.4. Samples are then degassed before analysis and injected into the sample cell of the Malvern (formerly MicroCal) VP-DSC (North Hampton, MA). The dialysis buffer is added to the reference cell of the calorimeter, and a buffer scan is used as the baseline for all experiments. The samples are scanned from 15°C to 100°C at a rate of 60°C/hour in duplicate. The transition midpoint (T_m) of the protein is determined by analysis of the data using the Origin 7.0 software. Data generated is used to internally to qualify the lot.

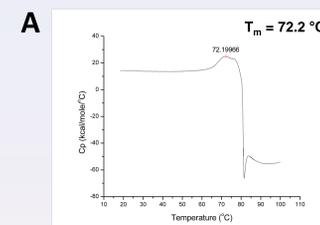
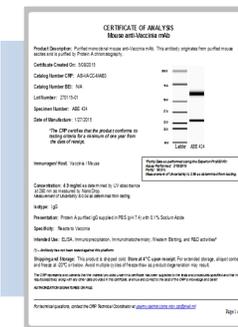


Figure #3. A. Example of the T_m graph. B. The DSC instrument.

Certificate of Analysis



A certificate of analysis is generated for each antibody sample that has successfully passed established specifications. With the Critical Reagents Program approval, data generated over the course of testing is reported on the CofA. A CofA is sent with each customer shipment.

Joint Acquisition Chemical Biological Radiological Nuclear (CBRN) Knowledge System (J.A.C.K.S.)

Users are able to view a live version of the database in the "CRP team room" on J.A.C.K.S. The creation of this database marks the successful transition of robust scientific data developed at ECBC through funding by the Defense Threat Reduction Agency (DTRA) to the Joint Project Management Office's Chemical Biological Medical System's (CBMS) Critical Reagents Program.



Acknowledgements

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