RB714, RB715, RB716, RB717 and RB718 antibodies recognize the histone HMtA protein from Methanobrevibacter smithii by ELISA

Philippe Hammel, Horst Pick, Duncan Bruce Sutherland

EPFL, ENAC Faculty, Station 6, CH-1015 Lausanne, Switzerland
Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB714, RB715, RB716, RB717 and RB718 detect by ELISA the *M. smithi* histone HMtA protein fused to a GST.

Introduction

HMtA (UniProt #A5UJP0) together with the HMtB protein form the histone-related protein complex (HMt). This complex has been shown to be able to bind and compact DNA to form nucleosome-like structures that contain positive DNA supercoils (Tabassum *et al.*, 1992). Here we describe the ability of five recombinant antibodies (RB714, RB715, RB716, RB717, RB718) to detect by ELISA a GST-fused HMtA protein.

Materials & Methods

Antibodies: ABCD_RB714, ABCD_RB715, ABCE_RB716, ABCD_RB717 and ABCD_RB718 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al.*, 2014) and produced as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc (RRB714, RRB715, RRB716, RRB717 and RRB718). HEK293 suspension cells (growing in HEK TF medium, Xell#861-0001, supplemented with 0.1% Pluronic F68, Sigma#P1300) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~50-80 mg/l) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the almost full-length HMtA protein (missing the initial methionine residue). This chimeric GST-HMtA was used as antigen for ELISA detection. As a negative control, an irrelevant GST-fused peptide (amino acids 916 to 999 of *Drosophila melanogaster* Spargel protein; UniProt # Q8IPM1) was used.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing in vivo N-biotinylated GST proteins were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma #A8275, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RRB714, RRB715, RRB716, RRB717, RRB718 bound in a concentration-dependent manner to the GST-HMtA antigen, but not to the GST-fused peptide negative control (Fig. 1).

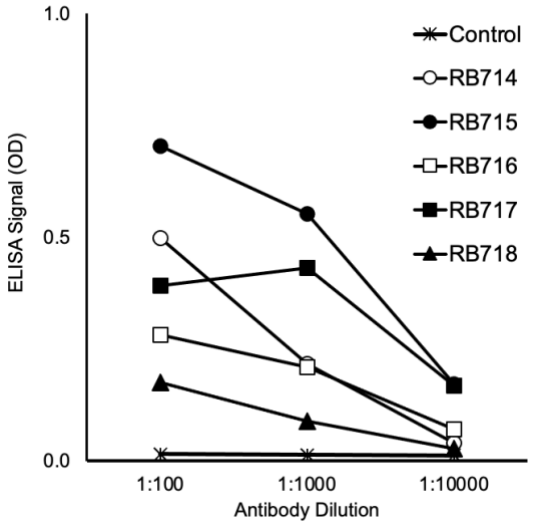

Fig. 1. Specific binding of RRB antibodies to the target GST-HMtA protein, but not to the GST-fused peptide negative control (shown only for RRB714, background curve is superimposed), as detected by ELISA.

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Tabassum R, Sandman KM, Reeve JN. HMt, a histone-related protein from *Methanobacterium thermoautotrophicum* delta H. J Bacteriol. 1992 Dec;174(24):7890-5. PMID:1459937

Conflict of interest

The authors declare no conflict of interest.