RB172 and RB173 antibodies recognize a fragment of the Dictyostelium discoideum *CMF protein by ELISA*

Philippe Hammel[1], Jean Pieters[2]

[1] *Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Biozentrum, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland*

Abstract
The recombinant antibodies RB172 and RB173 detect by ELISA a fragment of the *Dictyostelium discoideum* Conditioned Medium Factor (CMF) fused to a GST protein.

Introduction
The Conditioned Medium Factor (CMF, gene *cmfA*, Uniprot #P34090, DDB_G0275007) is a secreted protein involved in cell density sensing in *Dictyostelium discoideum* (Gomer *et al.*, 1991; Mehdy and Firtel, 1985). Here we describe the ability of two recombinant antibodies (RB172 and RB173) to detect by ELISA a GST-fused fragment of the CMF protein.

Materials & Methods
Antibodies: ABCD_RB172 and ABCD_RB173 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB172 and MRB173). HEK293 adherent cells (growing in DMEM, Gibco #11960044 supplied with 8% FBS) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~1-5 mg/l) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 99-154 of the CMF protein (`KTSLSSSKSESEHKSKFYKVIKSIYQTPTVTNGSFGIDGATTPSFSLTWDKPVVG`). This chimeric GST-CMF was used as antigen for ELISA detection. GST was used as negative control.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 MRB 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-mouse IgG (Bio-Rad #170-6516, 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 RB172 and, to a lesser extent, RB173 bound in a concentration-dependent manner to the GST-CMF antigen, but not to the GST negative control (Fig. 1). Note that this antigen only encompasses a small portion of the CMF protein, and that it is presumably not properly folded. Being produced in bacteria, it also lacks several post-translational modifications (disulfide bridges, glycosylations) typical of secreted proteins. Further experiments will be necessary to determine if and in what conditions these antibodies recognize the full CMF protein.

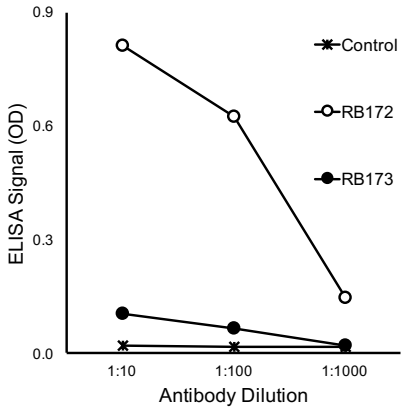

Fig. 1. Specific binding of RB antibodies to the target GST-CMF protein, but not to GST (shown only for RB172; RB173 background curve is superimposed), as detected by ELISA.

References
Gomer RH, Yuen IS, Firtel RA. A secreted 80 x 10(3) Mr protein mediates sensing of cell density and the onset of development in *Dictyostelium*. Development. 1991; 112:269-78. PMID:1663029

Mehdy MC, Firtel RA. A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in Dictyostelium discoideum. Mol Cell Biol. 1985; 5:705-13. PMID:2985966

Conflict of interest
The authors declare no conflict of interest.