

# RB014, RB015 and RB016 antibodies recognize a peptide of the *D. discoideum* Phg1A protein by western blot

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## Abstract

Recombinant antibodies RB014, RB015 and RB016 detect by western blot a peptide of the *Dictyostelium discoideum* Phg1a protein fused to a GST protein.

## Introduction

Phg1a (Phagocytic receptor 1a, DDB\_G0267444, UniProt #Q55FP0) is a protein belonging to the Transmembrane 9 superfamily, involved in adhesion, phagocytosis and bacterial killing in *D. discoideum* (Cornillon *et al.*, 2000). Here we describe the ability of the RB014, RB015 and RB016 antibodies to detect by western blot a fragment of the Phg1a protein fused to a GST protein.

## Materials & Methods

**Antibodies:** ABCD\_RB014, ABCD\_RB015 and ABCD\_RB016 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al.*, 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB014, MRB015 and MRB016). HeLa cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~1 mg/L) were collected after 4 days.

**Antigen:** The antibodies were originally raised against a GST protein fused to 91 residues of the extracellular N-terminal domain of the Phg1a protein (GEEGAIKVKNKITSVHTQIPYKYYQLPGVCQPKEGIIDDTENLGEILLGDRIENS DYT FNFLTDGGKCKVINS E SCSPIIKKEDLKVLEDRI).

This chimeric GST-Phg1a protein was used as antigen for detection. GST was used as a negative control.

**Protocol:** Expression of the GST-Phg1a recombinant protein was induced in *E. coli* bacteria growing exponentially (OD<sub>600</sub>, 0.5) at 37°C (in 50 ml of Luria-Bertani (LB) medium containing 20% glucose and 100 µM ampicillin) by addition of 1.5 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (4 ml of PBS + 1% Triton X100 + aprotinin 10 µg/ml + leupeptin 20 µg/ml + iodoacetamide 1.8 mg/ml + PMSF 18 µg/ml) and lysed by sonication. GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 500 µl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (v/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 15 µL of each sample was migrated (200 V, 30 min) in a 12% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1043), and transferred to a nitrocellulose

membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight at 4 °C in PBS containing 0.1% (v/v) Tween20 and 5% (w/v) milk, and washed three times (5 minutes) in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (undiluted), for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse (Biorad #170-6516, dilution 1:3000) for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) using a PXi-4 gel imaging systems (Syngene).

## Results

RB014, RB015 and RB016 antibodies specifically recognize the GST-Phg1a fusion protein (~35 kDa), as well a higher molecular weight product at ~42 kDa. The antibodies do not bind the GST negative control (Fig. 1). The RB015 antibody also detects the full-length endogenous *D. discoideum* Phg1a protein by western blot (Blanc *et al.*, 2014).

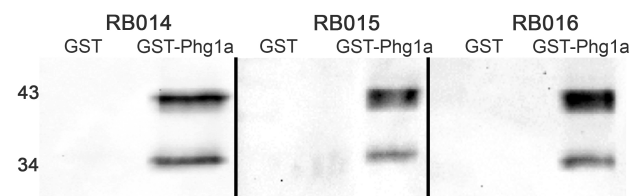


Fig. 1. Specific binding of RB014, RB015 and RB016 antibodies to the GST-Phg1A protein (predicted molecular mass ~35 kDa).

## References

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## Conflict of interest

The authors declare no conflict of interest.