Assay development for essential enzyme activity in the tegument of live Schistosomes

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- **Absorbance-based assays assess activity of indispensable *Schistosoma* enzymes**
- **Effectiveness of new drugs and vaccines can be measured in live parasites and in real-time**
- **Absorbance-based microplate readers by BMG LABTECH reliably read the assay in endpoint and kinetic mode**

Introduction

Schistosomiasis is a parasitic disease that affects over 200 million people in tropical, developing nations, causing severe morbidity and over 300,000 deaths annually. Schistosomiasis is treated with a single drug and no vaccine is available.

New drugs and vaccines to control *Schistosoma* parasites can be developed by targeting mechanisms vital to the functioning of the organism and desirably result in impairment or, preferably, death of the parasite. Schistosomes possess a dynamic outer surface membrane called a tegument that mediates many of the parasites' fundamental biological processes such as nutrient acquisition and immunity. Accordingly, molecules associated with the tegument have been a focus of schistosomiasis drug and vaccine development over the past few decades.

In this application note, we selected three *Schistosoma* surface-associated enzymes that are indispensable to parasitic survival: alkaline phosphatase[1]; phosphodiesterase SmNPP-5[2] and an acetylcholinesterase[3]. The activity of these molecules on the surface of live and intact larval and adult *Schistosoma* can be assayed in real time using cultured parasites, providing a tool to assess the efficacy of drugs or vaccines targeting these enzymes.

Assay Principle

The enzymes phosphodiesterase smNPP-5, alkaline phosphatase and acetylcholinesterase are essential for *Schistosoma* existence. Active enzymes cleave model substrates to chromophores (Fig. 1). Absorbance of the chromophores is measured to report on enzyme activity.

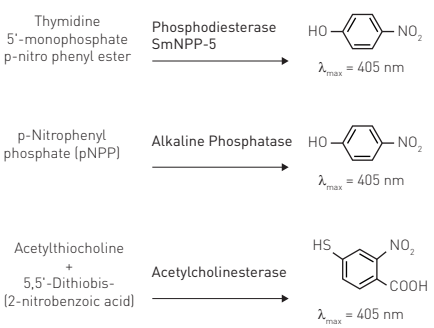

Fig. 1: Substrates and respective products for the three enzyme assessed in this study.

Materials & Methods

Assay components

Enzyme	Substrate	Assay buffer
Phospho-diesterase	Thymidine 5'-mono-phosphate p-nitrophenyl ester (0.5 mM)	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 60mM glucose, pH 8.9
Alkaline phosphatase	4-Nitrophenyl phosphate (2 mM)	0.1 M Glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4
Acetylcholin-esterase	Acetylthiocho-line (1 mM) 5,5'-Dithiobis (2-nitrobenzoic acid) 0.5 mM	0.1 M phosphate buffer, pH 7.4

Parasites were incubated in 0.5mL of enzyme-specific assay buffer (Table 1) in 48-well plates. Assays were performed in duplicate with various numbers of live larvae (schistosomula) (100, 200, 500, 1000, and 2000 per well) and adult worms (1, 2, 5 and 10 pairs per well) to estimate the number of parasite required for robust and reproducible results. Absorbance was measured with the FLUOstar Omega® and the following settings.

Instrument settings

FLUOstar Omega		
Optic settings	Absorbance, plate mode kinetic	
	Wavelengths settings	405 nm
General settings	Number of flashes	20
	Settling time	0.5 s
Kinetic settings	Number of cycles	68
	Cycle time	900 s
Incubation	37 °C	

Results & Discussion

The kinetic data for acetylcholinesterase activity in live adult worms is depicted in Fig. 2 (conducted in duplicate). The rate of substrate processing increases with increasing worm number, all substrate being processed in two hours for 10 pairs of worms.

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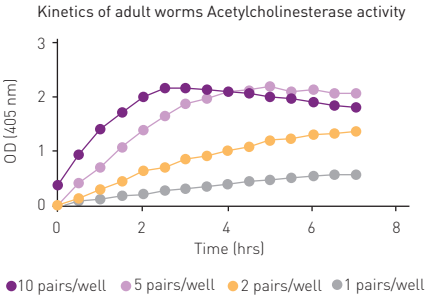

Fig. 2: Kinetic data for processing of acetylcholinesterase substrate by adult schistosomes.

A plot of absorbance values at 1 hour for acetylcholinesterase activity (Fig. 3) demonstrates a linear relationship between enzyme activity and worm number. This suggests that each live worm has similar concentrations of acetylcholinesterase in its tegument. Similar results were obtained for Alkaline Phosphatase and Phosphodiesterase activity in adult worms (data not shown).

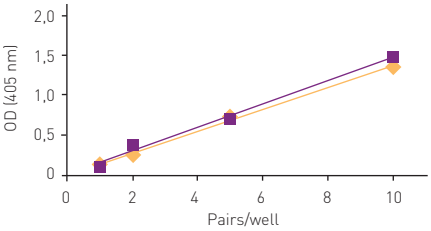

Fig. 3: Absorbance of acetylcholinesterase-processed substrate correlates with worm number after 1 hour of reaction (R^2>0.99). Duplicates are shown and demonstrate reproducibility.

A linear relationship was also obtained for live schistosomula when they were incubated with substrates for the three enzymes. Absorbance values at 17 hours for Phosphodiesterase (Fig. 4A) and at 4 hours for Alkaline phosphatase (Fig. 4B) also show a linear relationship between the rate of substrate processing and somule number (data for acetylcholinesterase not shown).

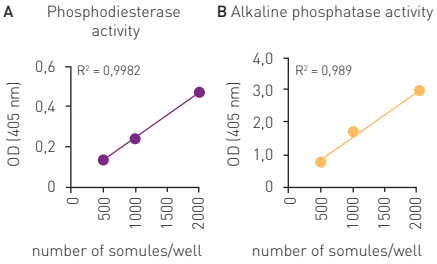

Fig. 4: Correlation of phosphodiesterase activity (absorbance at 405 nm) with number of schistosomula per well (in duplicate) after 17 hours (A). Correlation of alkaline phosphatase activity (absorbance at 405 nm) with schistosomula per well (in duplicate) after 4 hours (B).

Conclusion

This work establishes that adult schistosomes and the immature larvae can be used in a real-time assay for three crucial enzymes in the tegument of the parasites. This work will greatly aid in the screening and development of new drugs for the treatment of schistosomiasis. The work also highlights the versatility of the FLUOstar Omega microplate reader (BMG LABTECH) for the development of these assays and for drug development. Apart from fluorescence and luminescence capabilities, the FLUOstar Omega is equipped with a robust absorbance spectrometer, allowing fast and reliable endpoint and kinetic absorbance data.

References

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