

<b>Title</b>	<b>Leishmania EC50</b>		
<b>Protocol Number</b>	PARA19003	<b>Written by</b>	Duha A.
<b>Adapted from</b>	CFP Labs	<b>Version</b>	1.0
<b>Date created</b>	30 <sup>th</sup> September 2019	<b>Version update</b>	NA

### EC50 to validate drug resistance (promastigote)

Resistance is tested against the WT strain. The aim is to prove that the WT is sensitive to the drugs whereas the drug resistant strains resist all drug concentrations used

#### Protocol to perform EC50 in 24 well plate:

##### Sb resistant strain

- Set the parasite count to  $1 \times 10^6$  parasites per mL
- Add 2mL of Sb containing culture media to the first 12 wells
- Add 2mL of WT containing culture media to the next 12 wells
- Add SbIII solution (50mM) to all the wells following the template below

0 $\mu$ M	20 $\mu$ M (add 0.8 $\mu$ L)	40 $\mu$ M (add 1.6 $\mu$ L)	60 $\mu$ M (add 2.4 $\mu$ L)	80 $\mu$ M (add 3.2 $\mu$ L)	100 $\mu$ M (add 4 $\mu$ L)	Sb
0 $\mu$ M	20 $\mu$ M (add 0.8 $\mu$ L)	40 $\mu$ M (add 1.6 $\mu$ L)	60 $\mu$ M (add 2.4 $\mu$ L)	80 $\mu$ M (add 3.2 $\mu$ L)	100 $\mu$ M (add 4 $\mu$ L)	
0 $\mu$ M	20 $\mu$ M (add 0.8 $\mu$ L)	40 $\mu$ M (add 1.6 $\mu$ L)	60 $\mu$ M (add 2.4 $\mu$ L)	80 $\mu$ M (add 3.2 $\mu$ L)	100 $\mu$ M (add 4 $\mu$ L)	WT
0 $\mu$ M	20 $\mu$ M (add 0.8 $\mu$ L)	40 $\mu$ M (add 1.6 $\mu$ L)	60 $\mu$ M (add 2.4 $\mu$ L)	80 $\mu$ M (add 3.2 $\mu$ L)	100 $\mu$ M (add 4 $\mu$ L)	

- Reference range: 70.9 -85.4  $\mu$ M
- Sb reference range: > 100  $\mu$ M

### MF resistant strain

- Set the parasite count to  $1 \times 10^6$  parasites per mL
- Add 2mL of MF containing culture media to the first 12 wells
- Add 2mL of WT containing culture media to the next 12 wells
- Add MF solution (5mM) to all the wells following the template below

0 $\mu$ M	5 $\mu$ M (add 2 $\mu$ L)	10 $\mu$ M (add 4 $\mu$ L)	15 $\mu$ M (add 6 $\mu$ L)	20 $\mu$ M (add 8 $\mu$ L)	25 $\mu$ M (add 10 $\mu$ L)	MF
0 $\mu$ M	5 $\mu$ M (add 2 $\mu$ L)	10 $\mu$ M (add 4 $\mu$ L)	15 $\mu$ M (add 6 $\mu$ L)	20 $\mu$ M (add 8 $\mu$ L)	25 $\mu$ M (add 10 $\mu$ L)	
0 $\mu$ M	5 $\mu$ M (add 2 $\mu$ L)	10 $\mu$ M (add 4 $\mu$ L)	15 $\mu$ M (add 6 $\mu$ L)	20 $\mu$ M (add 8 $\mu$ L)	25 $\mu$ M (add 10 $\mu$ L)	WT
0 $\mu$ M	5 $\mu$ M (add 2 $\mu$ L)	10 $\mu$ M (add 4 $\mu$ L)	15 $\mu$ M (add 6 $\mu$ L)	20 $\mu$ M (add 8 $\mu$ L)	25 $\mu$ M (add 10 $\mu$ L)	

- Reference range: 12.05 -13.53  $\mu$ M
- MF reference range: > 25 $\mu$ M

### AmB resistant strain

- Set the parasite count to  $1 \times 10^6$  parasites per mL
- Add 2mL of AmB containing culture media to the first 12 wells
- Add 2mL of WT containing culture media to the next 12 wells
- Add AmB solution (270mM) to all the wells following the template below

0 nM	4 nM (add 0.03 $\mu$ L)	8 nM (add 0.06 $\mu$ L)	12 nM (add 0.089 $\mu$ L)	16 nM (add 0.12 $\mu$ L)	20 nM (add 0.15 $\mu$ L)	AmB
0 nM	4 nM (add 0.03 $\mu$ L)	8 nM (add 0.06 $\mu$ L)	12 nM (add 0.089 $\mu$ L)	16 nM (add 0.12 $\mu$ L)	20 nM (add 0.15 $\mu$ L)	
0 nM	4 nM (add 0.03 $\mu$ L)	8 nM (add 0.06 $\mu$ L)	12 nM (add 0.089 $\mu$ L)	16 nM (add 0.12 $\mu$ L)	20 nM (add 0.15 $\mu$ L)	WT
0 nM	4 nM (add 0.03 $\mu$ L)	8 nM (add 0.06 $\mu$ L)	12 nM (add 0.089 $\mu$ L)	16 nM (add 0.12 $\mu$ L)	20 nM (add 0.15 $\mu$ L)	

- WT reference range: 1.69 – 4.81 nM
- AmB reference range: > 20nM