PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (5) 905-910 May 2023

# Investigation of Some Oxidative Stress Parameters in *Drosophila Melanogaster* Exposed to Lead and Treated with *Picralima Nitida*

***OSUNBOR, JO; OROBOR, AD**

*Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria*

**Corresponding Author Email: oghogho.osunbor@uniben.edu; Tel: 08030752304*
*Co-Author Email: dianaorobor@gmail.com; Tel: 08108436663*

**ABSTRACT:** *Picralima nitida* is a plant used as herb in ethno-medicine for management of several health conditions. . *Drosophila melanogaster* is a species of fly referred to as the fruit fly. The objective of this study was to induce oxidative stress using lead in *Drosophila melanogaster* and to investigate its effects on the levels of selected oxidative stress parameters (malondialdehyde, nitric oxide and hydrogen peroxide) using standard method after dividing 250 flies into nine groups (A-I). Survival studies and biochemical assays were determined by spectrometry with the homogenized flies. The mean±SD of malondialdehyde, nitric oxide and hydrogen peroxide (90.40±.11.67, 0.29±0.04, 0.6978±0.04 respectively) were significantly lower in the control group than the flies induced with lead of different concentrations. When the lead induced flies were treated with *Picralima nitida* (10mg and 100mg), the level of malondialdehyde, nitric oxide and hydrogen peroxide were significantly reduced in all groups treated. Data from this study showed that lead at various concentrations has deleterious effects on the level on biomarkers (malondialdehyde, nitric oxide and hydrogen peroxide) in *Drosophila melanogaster.* While *Picralima nitida* had a modulatory effect on these oxidative stress parameters.

**DOI**: **https://dx.doi.org/10.4314/jasem.v27i5.3**



**Cite this paper as:** OSUNBOR, J. O; OROBOR, A. D. (2023). Investigation of Some Oxidative Stress Parameters in Drosophila Melanogaster Exposed to Lead and Treated with *Picralima Nitida*. *J. Appl. Sci. Environ. Manage.* 27 (5) 905-910

**Dates**: Received: 17 February 2023; Revised: 08 April 2023; Accepted: 16 April 2023
Published: 31 May 2023

**Key words**- *Picralima nitida;* oxidative stress biomarkers; *Drosophila melanogaster*

Lead is a ubiquitous environmental toxicant and heavy metal in the environment and a very strong poison. Lead poisoning is a serious and sometimes fatal condition. Some of the effects are permanent. In severe cases, anemia, seizures, coma, or death may occur. The primary cause of its toxicity is its predilection for interfering with the proper functioning of enzymes. It does so by binding to the sulfhydryl groups found on many enzymes (Rudolph *et al.*, 2003) or mimicking and displacing other metals which acts as cofactors in many enzymatic reactions (Dart *et al*., 2004). Lead toxicity arises from toxicants containing lead which may cause increase generation of Reactive Oxygen species (ROS) that interacts with the production of antioxidants and thus results in oxidative stress. Medicinal plants have been identified and used throughout human history. *Picralima nitida* possesses antioxidant properties and therefore could be used in the development of herbal medicine and/ or source of new molecules against some diseases (Campos *et al*., 2020). *Picralima nitida* (p. nitida) is one of the genus of Picralima. It is commonly called picralima, Akuamma or Pile plant and it belongs to the hunterieae tribe of apocynaceae family (Akinwuunmi *et al*., 2019). The plant is widely distributed in high deciduous forest of west-central Africa (Erharuyi *et al.*, 2014). *Picralima nitida* is a therapeutic herb used in ethno-medicine for the management of several disease conditions including diabetes. Various parts of the plant; the leaves, seeds, stem bark and roots are

**Corresponding Author Email: oghogho.osunbor@uniben.edu*

used for the treatment of fever, hypertension, jaundice, gastro-intestinal disorders and for malaria. *Drosophila melanogaster* is specie of fly in the family of drosophilidae (Ganiyu *et al*., 2018) and it's commonly referred to as fruit fly or vinegar fly. *Drosophila melanogaster* is typically used in research because it can be readily reared in the laboratory, has only four pairs of chromosomes, breeds quickly, and lays many eggs (Sang *et al.,* 2001). Other advantages are; 75% of the human genes implicated in diseases are conversed in *Drosophila*, with about 90% nucleotide sequence identified in some of its species (Reiter *et al.,* 2001). The short life span of these flies makes it possible to address lead metal toxicity and the modulatory roles of *Picralima nitida* during development and adulthood. Survival, neuronal function and behavior assays are easy to perform in this organism. It is also simple to search for toxicity-mediated mechanisms at the molecular level in Drosophila (Bonilla-Ramirez *et al.,* 2011). The aim of this study was to measure the levels of Malondialdehyde, nitric oxide, hydrogen peroxide in *Drosophila melanogaster* exposed to lead and treated with *Picralima nitida*.

## MATERIALS AND METHODS

*Drosophila melanogaster* stock culture; Wild- type fruit fly (Harwich strain) stock culture (originally from the National Species Stock Centre, Bowling Green, OH, USA) was obtained from Drosophila Laboratory, Department of Biochemistry, University of Ibadan, Oyo state, Nigeria. The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosed into young adult fruit flies under a natural photoperiod of about 12hour light and 12hour dark daily for the period of administration of the *Picralima nitida*. Flies were collected and separated into nine experimental groups with five vials of 50 flies in each group and the flies were then treated as stated below in the experimental design. The flies were fed with the standard formulated diet corn meal medium, which contained corn meal (52g), brewer's yeast (5g), glucose (3.5g), agar (7.5g) and Nipargin (1g), alcohol (1 ml). The water used for making the diet was distilled water Flies were randomly selected from vials. Caution was taken when counting the flies and an appropriate brush with soft ends was used. Much care was taken in handling the flies as to prevent "handling stress". Lead acetate used for the research was gotten from Drosophila Laboratory Central Research Laboratory, University of Benin, Edo state, Nigeria.

*Picralima nitida* fresh leaves were sourced from Upper Siluko, Egor, Benin-City, South -South, Nigeria and was validated at the Department of Plant Biology and Biotechnology (PBB), University of Benin. Aqueous extract of *Picralima nitida* leaves were processed by drying, pulverization, filtration and dehydration. For the survival assay, flies (both genders) were divided into nine groups, with each group having 3 vials each. Each vial contained 50 flies each with varied concentrations of *Picralima nitida* and lead acetate. The survival assay was carried out on three replicates of each concentration. The diet was changed every five days, during the period of this experiment. The survival rate was determined with all the concentrations, and both the live and dead flies were recorded daily. By the end of this experiment (14 days), the data obtained were accumulated and plotted as percentage of live and dead flies. The result was then compared with that of the control.

*Tissue Homogenate Preparation for Biochemical Assay:* For the determination of biochemical assays, a second group experiment was carried out. In this experiment, flies (both genders) were divided into nine groups, with each group having 5 vials each. Each vial contained 50 flies with varying concentrations of *Picralima nitida* and lead in each treatment vial relatively for a period of seven (7) days. At the end of the treatment period, flies were transfer into an empty treatment vial and immobilized using ice and then kept in an empty Eppendorf tube. It was weighed, homogenized using a homogenizing stick and 8.1M phosphate buffer (pH7.4) and then centrifuged at 4000xg for 10 minutes at $4^0$C (Allegra X-15R centrifuge, Beckman Coulter USA). Then, the supernatant separated into labelled Eppendorf tubes, and used for the various biochemical assays. All the assays were carried out in five replicates for the nine groups and relative absorbance read using Jenway spectrophotometer 7315, by Bibi Scientific Ltd, UK. Selected biomarkers (Malondialdehyde, nitric oxide and hydrogen peroxide) in the homogenized sample were determined. The amounts of nitrite in supernatants was measured following the Griess reaction (Green *et al*., 1982) by incubating a 250uL of sample with 250uL of Griess reagent [0.1% N-(1-naphthyl) ethylenediamine dihydrochloride; 1% sulfanilamide in 5% phosphoric acid; 1:1 punched at room temperature for 20 min. The absorbance at 550 nm (OD 550) was measured spectrophotometrically. Nitrite concentration was calculated by comparison with the OD 550 of a standard solution of known sodium nitrite concentrations. Hydrogen peroxide generation was determined according to the method of Wolff, 1994. The Malondialdehyde was estimated using the Gutteridge and Wikins (1982) method. Malondialdehyde is a product of lipid peroxidation which reacts with thiobarturic acid under heat to form a malondialdehdye-thiobarturic acid 2adduct, a pink

coloured complex measured with the at 532nm wavelength**.**

*Statistical Analysis:* All values were presented as mean ± standard error of mean (SEM) for fifty flies each for the nine groups. The significance of difference in the means of all the parameters were determined using one- way analysis of variance (ANOVA); 95% confidence interval). All statistical analysis was carried out using graph pad prism 5.0.

## RESULTS AND DISCUSSION

The curve shown above in figure 1 is a diagrammatic representation of the survival rate of the different dietary inclusion of *Picralima nitida* in *Drosophila melanogaster* fed with lead contaminated diets. Table 1 shows the comparison of the activity level of hydrogen peroxide, nitric oxide and malondialdehyde at different concentrations of lead acetate and P. nitida fed to D. melanogaster flies with control group. Flies fed with 1mg/dl lead acetate + 10mg/kg P. nitida had higher activity levels of hydrogen peroxide and malondialdehyde above the levels of control group while nitric oxide was significantly different ($p<0.05$). In the same vein, flies fed with 1mg/dl lead acetate and 100mg/kg P. nitida had the levels of slightly above the levels in the controls. Table 2 shows the comparison of the activity level of hydrogen peroxide, nitric oxide and malondialdehyde at different concentrations of lead acetate and P. nitida meal fed to D. melanogaster flies fed with 0.25mg/dl lead acetate had significantly higher activity of hydrogen peroxide (0.98±0.05 vs 0.69±0.04, $p<0.012$), nitric oxide (0.65±0.05vs 0.69±0.04, $p<0.012$) and lower activity of malondialdehyde (117.8±20.71 vs 0.69±0.04, $p>0.5$). Conversely, flies fed with 10mg/kg *Picralima nitida* has insignificantly lower activity levels of hydrogen peroxide, nitric oxide, malondialdehyde. Similarly, flies fed with 100mg/kg *Picralima nitida* had significantly higher activity of hydrogen peroxide, nitric oxide, malondialdehyde. However, flies fed 0.25mg/dl lead acetate + 10mg/kg had significantly higher activity levels of hydrogen peroxide (0.90±0.06 vs 0.69±0.04, $p<0.004$), nitric oxide (0.43 ±0.08vs 0.69±0.04, $p> 0.5$), malondialdehyde (93.08 ±23.51vs 0.69±0.04, $p>0.5$) were not significantly different. In the same vein, flies fed 0.25mg/dl lead acetate + 100mg/kg *Picralima nitida* had significantly hydrogen peroxide (0.72±0.03 vs 0.69±0.04, $p>0.5$) activity.

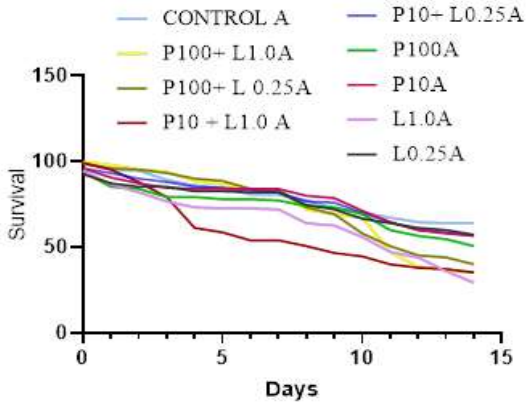


**Fig 1:** Survival curve showing the modulatory effects of dietary inclusion of *Picralima nitida* on survival rate in *Drosophila melanogaster* fed with lead contaminated diets.

The study showed that *Picralima nitida* was effective for the removal or reduction of Malondialdehyde, nitric oxide and hydrogen peroxide in Pb exposed *Drosophila melanogaster*. From the survival study (fig.1), 10mg/kg *Picralima nitida* had a higher survival rate of 85flies while 100mg/kg *Picralima nitida* concentration had a survival rate of 74flies. When compared to the control group with a survival rate of 96flies, it can be inferred that increased dose (100mg/kg) of *Picralima nitida* resulted in higher mortality rate. This is in agreement with Campos *et al*., (2020), which stated that despite the medicinal benefit of *Picralima nitida*, it has caused a toxic effect at high concentration in *Drosophila melanogaster*. Flies exposed to 0.25mg/dl lead acetate concentration showed little or no toxic effect on them having a survival rate of 86. But 1mg/dl Lead acetate concentration had deleterious effects on the flies with a very low survival rate of 44 and a high mortality rate of 106. This group of flies had the highest mortality in the study. This is in agreement with (Mathew and Krishnamurthy, *2018*) which states that the accumulation of lead at higher doses, within the flies might have induced deleterious effects on the larva resulting in reduced survival potential that might have decreased the rate of transforming to pupa.

**Table 1:** Comparison of measured Hydrogen peroxide, Nitric oxide, Malondialdehdye in different concentrations of Lead and P. Nitida meal with control

| **Treatment** | **Measured parameter** | | |
|---|---|---|---|
| | Hydrogen peroxide | Nitric oxide | Malondialdehyde |
| Group A Control (n=250) | 0.6978±0.04* | 0.29±0.04* | 90.40±.11.67* |
| Group B 1mg/dl lead Acetate (n=250) | 1.02±0.02[a] | 0.64±0.14[a] | 108.4±53.72* |
| Group G 1mg/dl lead Acetate+10mg/kg PN (n=250) | 0.78±0.07* | 0.42±0.04* | 104.6±12.17* |
| Group I 1mg/dl lead Acetate+100mg/kg PN (n=250) | 0.74±0.06* | 0.38±0.45* | 95.27±18.42* |

*Values are shown as Mean ± SD; a=0.0001; *=p>0.05: PN: PICRALIMA NITIDA*

**Table 2**: Comparison of measured Hydrogen peroxide, Nitric oxide, malondialdehyde in different concentrations of Lead and P. Nitida meal with control

| Treatment | Measured parameter | | |
|---|---|---|---|
| | Hydrogen peroxide | Nitric oxide | Malondialdehyde |
| Control (Group A) (n=250) | 0.69±0.04* | 0.29±0.04* | 90.40±.11.67* |
| Group B 0.25mg/dl lead Acetate (n=250) | 0.98±0.05[a] | 0.65±0.05[a] | 117.8±20.71* |
| Group C 10mg/kg PN (n=250) | 0.77±0.15* | 0.33±0.06* | 46.12±3.274* |
| Group D 100mg/kg PN (n=250) | 0.73±0.06* | 0.36±0.19* | 71.88±15.21* |
| Group F 0.25mg/dl lead Acetate+10mg/kg PN (n=250) | 0.90±0.06[b] | 0.43 ±0.08* | 93.08 ±23.51* |
| Group H 0.25mg/dl lead Acetate+100mg/kg PN (n=250) | 0.72±0.03* | 0.35±0.03* | 96.01±11.30* |

*Values are shown as Mean ± SD; a=0.012; b=0.004; *=p>0.5. PN: PICRALIMA NITIDA*

Lead also causes an irreversible neurobehavioral damage in many developing mammals that increases oxidative stress, which in turn leads to genetic manipulation, damage in neuronal DNA including apoptosis and Alzheimer's disease (Jaishankar *et al*., 2014). The effect of 10mg/kg *Picralima nitida* on flies exposed to 0.25mg/dl lead acetate concentration had ameliorative effects on the flies with a survival rate of 85 and a mortality rate of 65. 100mg/kg *Picralima nitida* on flies exposed to 0.25mg/dl Lead acetate concentration had little ameliorative effects on the flies with a survival rate of 60 and a high mortality rate of 90. But when varying concentrations of picralima nitda (10mg/kg and 100mg/kg) was administered on 1mg/dl Lead acetate exposed flies, it had very little ameliorative effects with a low survival rate of 53 and 57 respectively and a mortality rate of 97 and 93 respectively. From this study, it was seen that *Picralima nitida* in concentrations as low as 10mg/kg had the highest ameliorative effects on its own and when administered to treat Pb poisoning. However, *Picralima nitida* in concentrations as high as 100mg/kg also had ameliorative properties but not as effective as *Picralima nitida* in lower concentrations (10mg/kg).

**Table 3:** correlation of hydrogen peroxide, nitric oxide and malondialdehyde with concentrations of Lead Acetate

| Measured parameters | R-values | P-values |
|---|---|---|
| Hydrogen peroxide | 0.4917 | 0.4002 |
| Nitric oxide | -0.9411 | 0.0170* |
| Malondialdehyde | 0.3967 | 0.5085 |

**p<0.05. PN: PICRALIMA NITIDA; The activity levels of hydrogen peroxide (r=0.4917; p>0.4002), nitric oxide (r=-0.9411; p<0.0170) and malondialdehyde (r=0.3967; p>0.5085) correlated with lead acetate concentrations in table 3.*

This study showed that varying graded concentration of lead had a significant (p< 0.05) effect on hydrogen peroxide level when compared to the control group. There was no significant (p>0.05) effect on the hydrogen peroxide level after the infusion of the varying concentration of *Picralima nitida*, when compared to the control group. After infusing 0.25mg/dl and 1mg/dl Lead acetate to the flies that were previously fed with 10mg/kg *Picralima nitida*, a significant (p<0.05) effect was seen on the hydrogen peroxide. There was significant (p<0.05) effect after infusing 10mg/kg *Picralima nitida* to flies that were previously fed with 1mg/dl Lead acetate, however a significant difference (p<0.05) was seen in the level of hydrogen peroxide when the concentration given was 1mg/dl Pb-induced *Drosophila melanogaster* was increased to 100mg/kg *Picralima nitida*. High concentration of Hydrogen peroxide is produced by inflammatory and vascular cells and induces oxidative stress that may contribute to atherosclerosis and endothelial dysfunction. Hydrogen peroxide and Malondialdehyde are biomarkers of oxidation. (Shah et al., 2014). There was no significant (p>0.05) effect on the level of nitric oxide after infusing the flies with the varying concentration of Lead acetate and *Picralima nitida* separately when compared to the control group. No significant (p< 0.05) difference was seen in the level of nitric oxide when varying concentrations of *Picralima nitida* was administered to the flies that was previously fed with varying concentrations of Lead acetate. Nitric oxide is an inflammatory biomarker in oral and systemic diseases. Nitric oxide is widely considered one of the most important molecules produced in the human body, acting as a necessary regulator in a vast array of vital physiological functions, namely, blood pressure, immune response, and neural communication. It is identified in animal models that oxidative stress plays a significant role in the development of hypertension, in part by inactivation of Nitric oxide (Ghosh et al., 2004). There was no significant (p>0.05) effect on the level of Malondialdehyde when compared with the control group, after infusing varying concentration of Lead acetate in the flies. No significant (p>0.05) effect on Malondialdehyde after infusing varying concentrations of *picralima nitda* when compared to the control group. There was an effect on the level of Malondialdehyde after varying concentrations of *Picralima nitida* was administered to the flies that had been given varying concentration of Lead acetate. But it was not significant p>0.05. However, there was no significant difference (p>0.05) on malondialdehyde fed with 1mg/dl Lead when compared with dietary

inclusions of 1mg/dl Lead and 100mg/kg *Picralima nitida*. This means that *Picralima nitida* in high concentration has an ameliorative effect on lead induced Malondialdehyde level. The effects of Malondialdehyde is highly toxic fatty acid (polyunsaturated fatty acids) and it is a biomarker for various diseases patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer. It can result in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease. Therefore in this study, it showed that the levels of hydrogen peroxide in the control group was significantly lower in control and 100mg/kg *Picralima nitida* than those containing varying concentrations of *Picralima nitida* and Lead acetate treatment and the levels of nitric oxide in the control group was significantly lower in control and 10mg/kg *Picralima nitida* than those containing varying concentrations and whereas, the levels of Malondialdehyde was significantly lower in 10mg/kg *Picralima nitida* and control than those containing varying concentrations treatments. From this study, it can be deduced that the survival rate of *Drosophila melanogaster* reduced with increasing concentration of Lead acetate administered. The increased mortality rate could be attributed to oxidative stress caused by Lead acetate. Also, oxidative stress biomarkers (Malondialdehyde, nitric oxide and hydrogen peroxide) measured after giving various lead concentrations increased. Dietary inclusion of low concentration *Picralima nitida* in Pb- exposed *Drosophila* meals prolonged the survival rate and modulated the levels of the selected biomarkers (Malondialdehyde, nitric oxide and hydrogen peroxide) in the flies.

*Conclusion:* From this study it was seen that introduction of lead into the diets of the flies increased the levels Malondialdehyde, nitric oxide and hydrogen peroxide in the flies. The study also demonstrated that *Picralima nitida* may play a modulatory role in alleviating the effects of oxidative stress in the flies.

## REFERENCES


Akinwunmi, KF; Amadi, CH (2019). Assessment of antioxidant and antidiabetic properties of *Picralima nitida* seed extracts. *J. Med Plants Res.* 13(1): 9-17.

Bonilla-Ramirez, L; Jimenez-Del-Rio, M; Velez-Pardo, C (2011). Acute and chronic metal exposure impairs locomotion activity in Drosophila melanogaster: a model to study Parkinsonism. *Biometals* 24:1045–1057.

Campos, CD; Modupe, LP; Iheagwan, FN; Rotimi, OS; Chinedu, SN. (2020). Phytochemical Composition, Antioxidant activity and Toxicity of Aqueus Extract of *Pricalina natilda* in *Drosophila melanogaster*. *Trop. J. Nat. Prod. Res*. 4(12): 1147-1153.

Dart RC; Hurlbut, KM; Boyer-Hassen LV (2004). "Lead" In Dart, RC. Medical Toxicology, 3rd edition. Lippincott Williams and Wilkins. p. 1426.

Erhanruyi, O; Falodun, A; Langer, P (2014). Medicinal Uses, phytochemistry and pharmacology of *Picralima nitida* (Apocynaceae) in tropical diseases: A review. *Asian Pac J Trop Med.* 7(1): 1-8.

Ganiyu, O; Opeyemi, BO; Mopelola, YO; Seun, FA (2018). Effect of dietary inclusions of bitter kola seed on geotactic behavior and oxidative stress markers in *Drosophila melanogaster*. *Food Sci. Nutri*, 6(8):2177-2187.

Ghosh, M; Wang, HD; McNeill, JR (2004). Role of oxidative stress and nitric oxide in regulation of spontaneous tone in aorta of DOCA-salt hypertensive rats. *Br.J pharmacol*, *141*(4): 562-573.

Green, LC; Wagner, DA; Glogowski, J; Skipper, PL; Wishnok, JS; Tannenbaum, SR (1982) Analysis of nitrate, nitrite and 15N in biological fluids. *Anal. Biochem.* 126: 131–136.

Gutteridge, JM; Wilkins, S (1982). Copper-dependent hydroxyl radical damage to ascorbic acid: formation of a thiobarbituric acid-reactive product. *Febs Letters*, 137(2): 327-330.

Jaishankar, M; Tseten, T; Anbalagan, N; Mathew, BB; Beeregowda, KN (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip toxicol.* **7**:60-72.

Mathew, BB; Krishnamurthy, NB (2018). Assessment of lead toxicity using Drosophila melanogaster as a model. *J. Clin Toxicol*, 8(2), 380.

Reiter, LT (2001). A Systematic Analysis of Human Disease-Associated Gene Sequences in Drosophila melanogaster. *Genome Res.*, 11(6), 1114–1125.

Rudolph, MJ; Wuebbens, MM; Turque, O; Rajagopalan, KV; Schindelin, H (2003).Structural studies of molybdopterin synthase provide insights

into its catalytic mechanism. *JBC*, 278(16):14514-14522.

Sang, JH (2001). Drosophila melanogaster: the fruit fly. *Encyclopedia of genet*, 157, 162.

Shah, D; Mahajan, N; Sah, S; Nath, KS; Paudyal, B (2014). Oxidative stress and its biomarkers in systemic lupus erythematosus. *J. Biomed. Sci.* 21(1), 1-13.

Wolff, SP (1994). Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. In: Methods in enzymology Vol. 233, Academic Press, Cambridge, p.182-189.