Enzyme activity measurement (propyllaurate units)

Method:
This method describes the procedure to determine the activity of enzyme in propyllaurate units per gram enzyme (PLU/g).

Chemicals:
Lauric acid (99.5% acros 200.32 g / mol)
1-Propanol (Merck)
Demineralised water
Neutralized ethanol
0.1 M Sodium hydroxide in demineralised water
Thymolphthalein as indicator
Premixed 1-propanol with water (90 ml 1-propanol + 8 ml water)

Equipment:
Thermostated shaker/incubator. Magnetic stirrer.

Substrate:
For each sample the following substrate composition is used:
200 mg lauric acid (= 1 mmol)
90 microliter (= 1.2 mmol) 1-propanol
8 microliter water

Procedure:
1. The heating bath is thermostated at 60°C.
2. Lauric acid is weighed into the vial.
3. Then 98 µl of the 1-propanol-water mixture is added to the vial.
4. 5 mg catalyst is weighed on a paper and added to the vial.
5. The vial is closed and put in the heated shaker cell.
6. After 30 seconds the lauric acid is melted and the clock is started.
7. After 5 minutes 1.5 ml ethanol is added and after mixing the solution is pipetted into a 50 ml beaker **without** the catalyst.
8. The catalyst is washed 2 x with 2 ml ethanol which is also added to the 50 ml beaker, without the catalyst
9. A spoon tip of thymolphthalein and a stirrer bar is added to the solution.
10. The solutions are titrated with 0.1 M NaOH until the solution turns blue (at pH 10.0) and the acid values are calculated.
11. The acid value of 200 mg substrate is also determined separately

Calculation:

$$\text{Activity} = \frac{\text{ml sample} - \text{ml blank}}{W * t} * 100 = \text{PLU/g}$$

with W = grams of enzyme
t = time (5 minutes)
ml sample = titrated volume with enzyme
ml blank = titrated volume without enzyme