



## Aminoacyl tRNA synthetase

**Description:**

Aminoacyl-tRNA Synthetase, suspended in 10 mM Tris buffer (pH 7.6) containing 50% glycerol, 10 mM MgCl<sub>2</sub>, 10 mM KCl and 30 mM 2-mercaptoethanol.

**Activity:**

Approximately 20 U/μl

**Lot:**

# 050611

**Source:**

Bovine liver

**Storage conditions:**

-20<sup>o</sup> C.

**Application:**

This enzyme is used to catalyze the aminoacylation of tRNAs with its correspondent amino acid. Testing indicates that this enzyme can catalyze the aminoacylation of **lysine, glutamic acid, proline and tryptophane** with their correspondent tRNA. Aminoacylation of other amino acid-tRNAs should be tested.

**Unit definition:**

One unit will activate and attach 0.1 picomole of H<sup>3</sup>-labeled lysine amino acid to tRNA in 30 minutes at 37<sup>o</sup> C. 5 units of this enzyme can suffice for a 60μl reaction volume.

**Shipping conditions:**

Ice pack.

***Please see next page!***

**Protocol:**

The aminoacylation reaction (final volume up to 60 $\mu$ l) is carried out in a buffer containing:

- 50mM Tris-HCl, pH 7.6
- 50mM KCl
- 2 mM DTT
- 4mM ATP
- 20mM Mg(Ac)<sub>2</sub>
- 0.017mCi/ml <sup>3</sup>H-lysine (specific activity 80-110Ci/mmol, Perkin Elmer)
- 5 U bovine liver aminoacyl tRNA synthetase (Bio S&T, Canada)

The reaction is initiated by adding a maximum of 10 $\mu$ l of sample (about 10 pmole of tRNA). Incubate the mixture at 37<sup>o</sup>C for 30 minutes. A 2 $\mu$ l aliquot (or all of the 60 $\mu$ l reaction solution depending on the intensity of radioactivity) is mixed with 300 $\mu$ l of 5% TCA (cold). The tRNA is precipitated by incubating the mixture at 4<sup>o</sup>C for 30 minutes. The resulting solution containing the precipitate is filtered with Whatman glass fiber filter, and washed 3 times in 5% TCA and one time 100% alcohol or acetone. The filter is then air-dried and radioactivity measured in a scintillation counter.

***Note: ATP and DTT cannot be autoclaved; the solution should be prepared with sterilized water. ATP solution should be neutralized before making up to the final volume.***

**EXAMPLE**

An easy way to prepare the reaction:

Tris 0.5 M, pH 7.6	6 $\mu$ l
KCl 1 M	3 $\mu$ l
DTT 1M	0.12 $\mu$ l
ATP 0.1 M	2.4 $\mu$ l
Mg(Ac) <sub>2</sub> 0.5 M	2.4 $\mu$ l
Aminoacyl tRNA synthetase	5U*
Water	Make up total volume to 60 $\mu$ l*
tRNA sample	1 $\mu$ l*
<sup>3</sup> H-lysine (1mCi/ml)	1 $\mu$ l

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\*: variable volume