

SDS-PAGE Sample Loading Buffer 1×

(Reduction)

Introduction:

SDS-PAGE(SDS-PAGE Sample Loading Buffer, 1X), is an improved protein buffer dye on Bromphenol blue. It can be used directly for cell or tissue sample lysis, and follow-up routine SDS-PAGE sample Loading. The advantage of use SDS-PAGE Sample Loading Buffer (1X) is more convenient; the disadvantage is the lysate sample can not use for routine Bradford or BCA proteinconcentration method. Homogeneity of protein samples is difficult to control. We need to use Coomassie brilliant blue staining results or Western test results to adjust the sample size. When the cell or tissue can be controlled homogeneous, it will be more convenient to obtain protein samples by direct pyrolysis of the lysate.

The product can also use to dilute SDS-PAGE sample waiting for loading.

Contents:

Cat No.: BD0034-1

SDS-PAGE Sample Loading Buffer (1X) (Reduction) 15ml

Procedure:

1. Dissolve SDS-PAGE Sample Loading Buffer, (1X) at room temperature or in water bath less than 37° C.

2. For Adherent cells:Remove the inoculum and wash it once with PBS, saline or serum-free culture medium (if there is no interference with the protein in the serum, it can not be washed). Add the lysate according the ratio of 6 hole plates added to each hole with 150-250ul SDS-PAGE Sample Loading Buffer (1X). Blow it down with the gun, made SDS-PAGE Sample Loading Buffer (1X) fully contacted with cells. Usually the cells will be splitting after 1-2s. Samples after pyrolysis were collected in a clean centrifuge tube.

3. For suspension cells: Centrifugally collect cells and use fingers to force cells to disperse. Add the buffer according the ratio of 6 hole plates added to each hole with 150-250ul SDS-PAGE Sample Loading Buffer (1X). Use fingers to flick the cells. There should be no obvious cell precipitation after full lysis. If there are more cells, it must be packed into 50-100 thousands of cells / tubes before splitting.

4. For tissue samples:

a. Cut the tissue into tiny pieces.

b. Add the buffer according the ratio of every 20ug with 150-250ul SDS-PAGE Sample Loading Buffer (1X). (If the cracking is not enough, can add more SDS-PAGE Sample Loading Buffer (1X), if a high concentration of protein is needed, can reduce the amount of SDS-PAGE Sample Loading Buffer (1X).

c.Homogenate with glass homogenizer until full lysis.

d. After full cracking, the samples were collected into a clean centrifuge tube.

Explanation: If the tissue sample itself is very small, it can be sheared properly and cracked directly with pyrolysis solution. The sample can be fully cracked by strong vortex. The advantage of direct pyrolysis is that it is more convenient and does not need to use homogenizer. The disadvantage of direct pyrolysis is that it is more sufficient than that of homogenizer.

5. Heat 100 $\,^{\circ}$ C or boiling water for 5-10 minutes to make full denatured protein. Explanation:

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Before boiling, a viscous translucent substance is usually found in the protein sample. Usually, the viscous translucent substance disappears after boiling in the boiling water bath for 8-10 minutes in the buffer of the sample for subsequent operation.

Note: If the initial dosage of cells or tissues is large and the genomic DNA content is high, it may still be viscous or viscous translucent after boiling for 5-10 minutes. At this time, it is need to boil for 5-10 minutes or add a proper amount of 1X buffer before boiling for 3-5 minutes. After boiling, the proteins bound to genomic DNA can be released sufficiently. At the same time, it will lead to partial breakage of genomic DNA, which will make the sense of stickiness disappear. This will not affect the subsequent operation.

6. After cooling to room temperature, centrifuge slightly at room temperature to precipitate possible impurities and so on. The supernatant can be directly sampled into the SDS-PAGE additive hole. Usually electrophoresis is stopped when the blue dye reaches the bottom of the gel.

Attention:

SDS-PAGE Sample Loading Buffer (1X) contains a small amount of mercapto ethanol, has a slight irritating smell.

SDS-PAGE Sample Loading Buffer (1X) should be used after dissolving completely

The product is limited to the scientific research of professionals. It can not be used for clinical diagnosis or treatment. It can not be used for food or medicine. It can not be stored in ordinary houses.

For your safety and health, please wear test clothes and disposable gloves.

Storage & Shelf life:

Store at -20 $^\circ\!\mathrm{C}\,$, Period of validity for one years .

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