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Product information

QF 24 V4
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Phenol Solutions

Catalog #: PC5523 / PC6910 / PC6920
Size: 100 ml, 400 ml
Storage: Check storage conditions*

*: Storage temperature varies, check storage conditions for individual product code.

Product Description:

Even though Phenol (catalogue #PB4112 / #PD0252) appear as a colorless-to-white solid when pure, phenol statured solutions manufactured by Bio Basic Inc. appear as bright yellow in color. This is because, an antioxidant, 8-Hydroxyquinoline has been added to organic extraction buffers that contain phenol (Kirby, 1956). As an antioxidant, 8-hydroxyquinoline stabilizes phenol and retards the formation of quinones (phenol oxidation products). It is usually added to a final concentration of 0.1% (w/v). 8-Hydroxyquinoline imparts a bright yellow color to the phenol:chloroform to which it is added, thereby helping the investigator keep track of the organic and aqueous phases during the nucleic acid purification process.

Kirby (1956) Biochem. J. 64:405

Storage Conditions:

Cat #	Description	Size	Storage
PC5523	Phenol-liquid, saturated with water	100 ml	4°C
PC6910	Phenol, saturated (pH7.9)	100 ml, 400 ml	2-8°C
PC6920	Phenol, saturated (pH4.5)	100 ml, 400 ml	4°C

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Protocols:

A: Tube Test Assay:

1. Perform assay at room temperature and use 2ml tubes for assay.

2. Prepare a set of protein standards using the supplied BSA Protein Standard solution. For example, 0, 8, 16, 24, 40, 50µg.

3. Add 1-50µl of each protein sample to be assayed to separate 2ml tubes.

NOTE: It is recommended that each sample be assayed in duplicate. The total amount of protein should not exceed 50µg and we recommend using various dilutions of each sample to ensure they are below 50µg.

NOTE: For determination of protein concentration in buffers free of interfering agents, please skip steps 4-7.

4. Add 0.5ml Precipitation Reagent I to each tube and vortex. Incubate for 2-3 minutes at room temperature.

5. Add 0.5ml Precipitation Reagent II to the tubes and vortex.

6. Centrifuge the tubes at maximum speed (~10,000xg) for 5 minutes to pellet the precipitated protein. For easier identification of the pellet, ensure all the tubes are centrifuged with the cap hinge facing outwards.

7. Decant the supernatant and remove residual liquid with a pipette, then invert the tube on clean filter paper and allow the liquid to completely drain from the tube.

Optional: To enhance washing for problematic samples, please see the Troubleshooting section.

8. Add 100µl Copper Solution (Agent I) and 400µl deionized water to each tube and vortex until the precipitated protein pellet dissolves.

9. Using a 1ml pipette, rapidly transfer 1ml Agent II directly into each tube and immediately mix by inverting the tubes.

10. Incubate at room temperature for 15-20 minutes and then immediately read absorbances at 480nm. The absorbance should be read within 10-20 minutes.

11. Plot absorbance against protein concentration to determine protein concentrations of each unknown.

NOTE: Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.

B: Microplate Test Assay:

NOTE: For high throughput 96-well assays, we recommend using 2ml deep round or V- bottom well titer plates. Protocol requires centrifugation of the 96-well plate at 5,000xg and this may require a special centrifuge adaptor.

1. For high throughput 96-well assay, follow steps 1-5 of the above Tube Test Assay protocol.

2. Centrifuge the 2ml deep round or V- bottom well titer plate at ~5,000xg for 7 minutes to pellet the precipitate. Discard the supernatant by inverting the titer plate. Allow the liquid to completely drain off the wells by placing the inverted titer plate on clean filter paper.

3. Continue with the above Tube test assay protocol following steps 8-9.

4. After incubation, transfer 200-250µl assay reaction product from each well into the corresponding well of a new flat bottom 96 well microplate and measure the absorbance at 480nm. The absorbance should be read within 10-20 minutes.

NOTE: Do not subtract blank reading from the sample reading as absorbance will decrease as protein concentration increases.

Troubleshooting:

Problems	Cause	Suggestions
Interference still occurring. (Readings very low and show limited change with increasing concentrations)	Interfering agents not fully removed due to carry over at step 7.	Ensure all supernatant is removed in step 7. There should be no residual liquid.
	Exceedingly high concentrations of interfering agents in starting samples.	If high concentrations of interfering agents are present, additional washing after step 7 may be required. Add 0.5ml Precipitation Reagent I and then 0.1ml Precipitation Reagent II to the pellet. Gently invert a few times without disturbing the precipitate and repeat steps 6-7.
A linear response between 0.5-50µg not visualized. The standard curve is not straight.	Poor centrifugation at Step 6. Failure to pellet all the protein.	Centrifuge for >5 minutes at >10,000xg.
	Insufficient mixing of Agent II	Use a 1ml pipette and rapidly shoot the Agent II directly into the tube. Immediately invert to mix.
Low protein concentrations.	Protein pellet not fully resuspended	Vortex pellet for 10-50 seconds at step 8.

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