

CARREZ CLARIFICATION ASSAY PROTOCOL

K-CARREZ

03/22

40-1000 Assays per Kit
(depending on application)



Megazyme

INTRODUCTION:

Proteins, emulsions, and turbidity can cause issues and interfere in many analytical determination procedures. This interference is most prevalent in food samples, as their extracts often contain high levels of protein and fat. The Carrez Clarification Kit provides easy, ready to use solutions which can remove these interfering compounds from samples, allowing improved handling and more accurate measurement of target analytes such as carbohydrates and alcohols.

The reagents supplied can be used in place of Concentrated Carrez Reagents in the Lactose Assay Kit ([K-LOLAC](#)) and the Lactulose Assay Kit ([K-LACTUL](#)). They can also be used (following dilution) in place of Carrez Reagents in a number of Megazyme kits including the Lactose/Galactose Assay Kit ([K-LACGAR](#)), L-Lactic Acid (L-Lactate) Assay Kit ([K-LATE](#)), D-/L-Lactic Acid (D-/L-Lactate) Assay Kit ([K-DLATE](#)) and Formaldehyde Assay Kit ([K-FRHYD](#)), among others.

PRINCIPLE:

Sample clarification is based on formation of a sparingly soluble solid zinc hexacyanoferrate(II) ($Zn_2[Fe(CN)_6]$). This is formed when Carrez solutions (I and II) are added into an aqueous environment. This solid attracts high molecular weight compounds such as proteins through adsorption, leading to precipitation of the complex. Precipitated complex is then removed through filtration or centrifugation, yielding a clarified sample solution.

INTERFERENCE:

Samples that contain or might be converted to citrate (aconitate), ascorbate and urea/ammonia will interact with Carrez reagents and should not be used in enzyme-based assays. Carrez reagents work best at pH 7.5 to 8, interference may occur when used outside of this pH range.

SAFETY:

Warning: Carrez I will liberate highly toxic cyanide gas if exposed to strong acids.

The general safety measures that apply to all chemical substances should be adhered to. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 40-1000 assays (sample type dependant) are available from Megazyme.

Bottle 1: Concentrated Carrez I (50 mL), potassium hexacyanoferrate (15% w/v).
Store at room temperature. See individual label for expiry date.

Bottle 2: Concentrated Carrez II (50 mL), zinc sulphate heptahydrate (30% w/v).
Store at room temperature. See individual label for expiry date.

PREPARATION OF REAGENTS:

These reagents can be used as supplied in place of concentrated Carrez Reagents in the following Megazyme kits:

K-LOLAC K-LACTUL

1. Use the contents of bottle 1 as supplied or as outlined in the relevant assay protocol.
2. Use the contents of bottle 2 as supplied or as outlined in the relevant assay protocol.

These reagents may also be used (following dilution) in place of Carrez Reagents in the following Megazyme kits:

K-LACGAR K-LATE K-DLATE K-FRHYD

1. Accurately dispense 25 mL of Concentrated Carrez I into a graduated cylinder and add distilled water to a final volume of 100 mL. Mix thoroughly before use. Use as outlined in the assay protocol or using the procedure below.

Stable for > 1 year at room temperature.

2. Accurately dispense 25 mL of Concentrated Carrez II into a graduated cylinder and add distilled water to a final volume of 100 mL. Mix thoroughly before use. Use as outlined in the assay protocol or using the procedure outlined below.

Stable for > 1 year at room temperature.

PREPARATION OF ADDITIONAL REAGENTS (not supplied):

1. **Sodium Hydroxide (NaOH, 100 mM)**

Dissolve 4 g of NaOH in 1 L of distilled water. Store at room temperature. Note that this reagent is not required for all sample clarification protocols.

EQUIPMENT (RECOMMENDED):

1. Micro-pipettors (100 μ L and 1000 μ L).
2. Vortex mixer.
3. Graduated cylinder (100 mL).
4. Glass beaker (100 mL).
5. Volumetric flask (100 mL).
6. Disposable 1.5 mL polypropylene microfuge tubes.
7. Microfuge (required speed 13,000 rpm).

ASSAY PROCEDURE:

If clarification is recommended as part of another Megazyme products assay protocol please refer to the products assay protocol for the sample clarification method. Alternatively, a generic procedure is outlined below:

1. Accurately weigh the sample (approx. 1-10 g, sample weight will depend on the method and analyte) into a 100 mL glass beaker and add approx. 60 mL of distilled water. Note that solid samples may require homogenisation or hot water extraction.
2. Add 5 mL of Carrez II solution and mix.
3. Add 5 mL of Carrez I solution and mix.
4. Measure the pH and adjust to pH \sim 7.5 if required using sodium hydroxide solution (100 mM).
5. Quantitatively transfer to a 100 mL volumetric flask and make to volume with distilled water. Mix thoroughly by inversion.
6. Transfer approx. 1 mL to a polypropylene microfuge tube and centrifuge at 13,000 rpm for 10 min. The resulting solution may then be used to analyse analyte concentrations in the following Megazyme kits [K-LOLAC](#), [K-LACTUL](#), [K-LACGAR](#), [K-LATE](#), [K-DLATE](#) and [K-FRHYD](#) or other assay procedures.
7. It may be possible (with some sample types) to filter the solution instead of centrifugation. To perform this, filter the solution through Whatman No. 1 (or equivalent) filter paper. Following filtration take the clear supernatant through to subsequent analyte determination steps.

NOTE: If sample clarification is not achieved using filtration through filter paper centrifugation is required.

CALCULATION:

The analyte concentration can be adjusted to take Carrez clarification into account as follows:

$$= \frac{c_{\text{analyte}} [\text{g/L sample solution}]}{\text{weight}_{\text{sample}} [\text{g/L extracted solution}]} \times 100 [\text{g/100 g}]$$

where:

c_{analyte} = Analyte concentration in g/L generated using Megazyme protocol

$\text{weight}_{\text{sample}}$ = $\frac{\text{Weight of sample extracted (g)}}{\text{Extract volume (L)}}$



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WITHOUT GUARANTEE

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