# ⟨165⟩ PREKALLIKREIN ACTIVATOR

## DEFINITION

Prekallikrein activator (PKA), also known as Factor XIIa, activates prekallikrein to kallikrein. Activated kallikrein cleaves kininogens into kinins (e.g., bradykinin) that can result in a loss of blood pressure. PKA is assayed by its ability to activate prekallikrein, which once activated cleaves a synthetic peptide substrate and releases a chromophore that can be measured spectrophotometrically. The amount of PKA is determined by comparison with reference standard material. The procedure presented in this chapter traditionally has been used to determine the amount of PKA in human plasma derivatives such as albumin and immunoglobulin preparations.

Some immunoglobulin preparations may contain kallikrein or kallikrein-like activity in the absence of the added prekallikrein substrate, resulting in turnover of the synthetic peptide substrate. If present, this kallikrein or kallikrein-like activity requires consideration when analysts verify the assay for suitability under the conditions of use. Some questions to consider when verifying the assay for suitability under the conditions of use include:

- Are sample matrix effects observed?
- Is the appropriate statistical model used for data analysis?
- Is an endpoint analysis sufficient, or is a kinetic analysis needed?

(For informational purposes only, refer to sections 2, 4, and 5 of *Design and Development of Biological Assays* ⟨1032⟩ when considering assay fitness for use.)

## ASSAY

• **PREKALLIKREIN ASSAY**

**Buffer A:** Prepare a solution of 0.05 M tris(hydroxymethyl)aminomethane and 0.15 M sodium chloride. Adjust with 2 N hydrochloric acid to a pH of 7.95–8.05.

**Buffer B:** Prepare a solution of 1.0 M tris(hydroxymethyl)aminomethane. Adjust with 2 N hydrochloric acid to a pH of 7.95–8.05.

**Solution A:** Prepare a solution of 0.33 N hydrochloric acid.

**Solution B:** Prepare a solution of 0.33 N sodium hydroxide.

**Solution C:** Prepare a solution of 50% acetic acid.

**Chromogenic substrate solution:** Prepare a solution of a suitable chromogenic substrate for amidolytic test specific for kallikrein in *Buffer A* to obtain a solution having a concentration of about 1.2 mM.

**Prekallikrein substrate:** Use a suitable *Prekallikrein substrate*. Test the *Prekallikrein substrate* for the absence of kallikrein activity by mixing 1 part with 20 parts of the *Chromogenic substrate solution* prewarmed at 37°, and incubate at 37° for 2 min. The substrate is suitable if the increase in absorbance at 405 nm is less than 0.001/min. [NOTE—To remove proteinase inhibitor contamination from the *Prekallikrein substrate*, acid treatment of the prekallikrein should be performed individually on each sample before use.] If frozen, thaw *Prekallikrein substrate* at 35°–39°. Add 2.0 mL of *Solution A* to 4 mL of *Prekallikrein substrate*, and incubate for 15 min at 23°–25°. Bring pH back to neutrality with 2.0 mL of *Solution B*. Add 200 µL of *Buffer B*, and store at room temperature for NMT 4 h.

**Standard solutions:** Dissolve the contents of a vial of USP Prekallikrein Activator RS in 0.5 mL of water. Dilute USP Prekallikrein Activator RS with *Buffer A* (1:5). Make five evenly spaced *Standard solutions* between 0 and 8.5 IU/mL.

**Sample solution:** Dilute sample appropriately to fall within the range of the standard dilutions with *Buffer A* performing at minimum (1:1).

**System suitability**

**Samples:** *Buffer A* and *Standard solutions*

**Suitability requirements**

**Assay range:** If the absorbance value of a sample is higher than the highest value of the standard curve, dilute the sample with *Buffer A* and repeat the *Assay*. If the absorbance of a sample is lower than the *Assay* limit of detection, the activity should be reported as less than the limit of detection.

**Correlation coefficient:** NLT 0.99

**Analysis**

**Samples:** *Buffer A*, *Standard solutions*, and *Sample solution*

[NOTE—This procedure also can be performed using alternative platforms. Care must be taken to minimize the time between the addition of *Prekallikrein substrate* to the *Standard solutions* and the *Sample solution* because some degradation of the *Prekallikrein substrate* can occur with time when thawed.]

To four separate individual wells of a suitable microtiter plate transfer 25 µL of either the *Standard solutions* or the *Sample solution*. Incubate the microtiter plate for 2–3 min at 35°–39°. Start to prewarm *Buffer A*, the *Prekallikrein substrate*, and the *Chromogenic substrate solution* at 35°–39°. For each *Standard solution* and *Sample solution*, in two of the four wells add 100-µL *Buffer A* and to the other two wells add 100-µL prewarmed *Prekallikrein substrate*; incubate at 35°–39° for 10 min. Add 125 µL of prewarmed *Chromogenic substrate solution* to all wells in the same order as the *Buffer A* and *Prekallikrein substrate* additions, and incubate at 35°–39° for 20 min. Stop the reactions with 50 µL of *Solution C*, and mix well. Read absorbance of each well of the plate at 405 nm within 2 h. Subtract the average $A_{405}$ of the two wells containing *Buffer A* for each *Standard solution* and *Sample solution* to give the corrected $A_{405}$ values. Using the least-squares method of linear regression, generate a standard curve from the activities of the *Standard solutions* (IU/mL) and their corresponding corrected absorbance values. Calculate prekallikrein activities of samples from the calibration curve of the standards. To obtain the corrected activity in IU/mL, multiply each activity by the sample's dilution factor.

## ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Prekallikrein Activator RS