Ab155901 Creatine Kinase Activity Assay Kit (Colorimetric)

For accurate measurement of creatine kinase activity in a variety of biological samples.
This product is for research use only and is not intended for diagnostic use.

Storage and stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 2 months.

Materials supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 20	25 mL	-20°C	-20°C
Creatine	1 mL	-20°C	-20°C
ATP I	1 vial	-20°C	-20°C
CK Enzyme Mix	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	-20°C
NADH Standard I	1 vial	-20°C	-20°C
Active Creatine Kinase	1 vial	-20°C	-20°C

PLEASE NOTE: ATP I was previously labelled as ATP (Lyophilized), and Assay Buffer 20 as Assay Buffer XX and CK Assay Buffer, and Developer Solution III as CK Developer (Lyophilized), and Active Creatine Kinase as Positive Control (Lyophilized), and Creatine as CK Substrate, and NADH Standard I] as NADH Standard (Lyophilized). The composition has not changed.

Materials required, not supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- (Optional) 10 kD Spin Columns (ab93349) – to remove small molecules from sample

Reagent preparation

Briefly centrifuge small vials at low speed prior to opening
Assay Buffer 20: Ready to use. Equilibrate to room temperature before use. Store at -20°C.
Creatine: Ready to use. If necessary, warm in 37°C water bath to dissolve any precipitate, then vortex to mix thoroughly. Aliquot substrate so that you have enough to perform the desired number of assays. Store at -20°C.
ATP I: Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Aliquot ATP I so that you have enough to perform the desired number of assays. Store at -20°C. Use within 2 months.
CK Enzyme Mix: Reconstitute with 220 µL Assay Buffer 20. Pipette up and down to dissolve completely. Aliquot enzyme mix so that you have enough to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice whilst in use.
Developer Solution III: Reconstitute with 220 µL ddH_2O. Pipette up and down to dissolve completely. Aliquot developer so that you have enough to perform the desired number of assays. Store at -20°C. Use within 2 months.

NADH Standard I: Reconstitute with 50 µL Assay Buffer 20 to generate 10 mM (10.0 nmol/µL) NADH Standard I solution. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C. Keep on ice while in use.
Active Creatine Kinase: Reconstitute with 200 µL Assay Buffer 20 to generate 10 mU/µL stock and mix thoroughly. Aliquot Active Creatine Kinase so that you have enough to perform the desired number of assays. Store at -20°C.

Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be prepared immediately prior use. Do not store for future use.

1. Prepare 1mM of NADH Standard I by adding 10 µL of 10 mM NADH Standard I to 90 µL Assay Buffer.
2. Using 1 mM standard, prepare standard curve dilution as described below in a microplate or microcentrifuge tubes:

Standard #	Volume of NADH Standard I (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

Sample preparation

General Sample Information

We suggest performing several dilutions to ensure readings are within the standard value range. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. If that is not possible, snap freeze samples in liquid nitrogen upon extraction and store immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest amount of cells necessary for each assay (initial recommendation = 2 x 106 cells).
2. Wash cells with cold PBS and resuspend cells in 100 µL ice cold Assay Buffer 20.
3. Homogenize cells quickly by pipetting up and down a few times.
4. Centrifuge sample for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a new tube and keep on ice.

Tissue samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).
2. Wash tissue in cold PBS and resuspend tissue in 100 µL of ice cold Assay Buffer 20.
3. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
4. Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a new tube and keep on ice.
6. Tissue samples (liver, for example) may contain small molecules such as ADP or NADH that generate background. Remove these molecules from sample by using a 10kD Spin Column (ab93349).

Plasma and Serum samples:

Plasma and serum samples can be tested directly by adding sample to the microplate wells.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

Assay procedure

- Equilibrate all materials and prepared reagents to correct temperature prior to use.
- We recommended to assay all standards, controls and samples in duplicate.

Plate Loading:

Standard wells = 50 µL standard dilutions

Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 20).

Sample Background control wells= 1 - 50 µL samples (adjust volume to 50 µL/well Assay Buffer 20). We recommend including a Sample Background control well for each sample tested.

Active Creatine Kinase (positive control) = 2 - 10 µL (adjust volume to 50 µL/well with Assay Buffer 20).

Reaction Mix:

Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number reactions +1).

Components	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 20	34	36
CK Enzyme Mix	2	0
Developer Solution III	2	2
ATP I	2	2
Creatine	10	10

Add 50 µL of Reaction Mix into each standard, sample and positive control sample wells and mix well.

Add 50 µL of Background Reaction Mix to Background control sample well and mix well.

Plate Measurement:

Measure output at OD 450 nm on a microplate reader in a kinetic mode, every 1 – 2 minutes, for at least 10 – 40 minutes at 37°C protected from light. Most reactions will occur within the first 10 minutes.

NOTE: *Incubation time depends on the Creatine Kinase (CK) Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T1 and T2) in the linear range (OD values A1 and A2 respectively) to calculate the CK activity of the samples. For standard curve, do not subtract A2 from A1. Standard curve can also be read in end point mode (i.e. at the end of incubation time).*

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
2. Standard curve calculation:
   Average the duplicate reading for each standard.
   Plot standard curve readings and draw the line of the best fit to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).
3. Measurement of CK activity in sample:
   Calculate ΔOD for sample as follows:

   ΔOD450nm = (A2 – A2BG) – (A1 – A1BG)

   A1 is the sample reading at time T1.
   A1BG is the background control sample at time T1.
   A2 is the sample reading at time T2.
   A2BG is the background control sample at time T2.
   Use the ΔOD450nm to calculate amount of NADH generated by CK during the reaction time (ΔT).
4. CK activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$\mathbf{CK\ Activity} = \left(\frac{\mathbf{B}}{\mathbf{\Delta T x\ V}}\right) * \mathbf{D}$$

B = Amount of NADH in sample well calculated from standard curve (nmol).
ΔT = Reaction time (minutes).
V = Original sample volume added into the reaction well (mL).
D = Sample dilution factor.
CK activity can also be expressed as U/mg of total protein in the sample.

Unit CK activity = amount of CK that will generate 1.0 µmol of NADH per min at pH 9.0 at 37°C.

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

FAQs

Which protein assay is compatible with this kit?

We suggest you use a detergent compatible BCA assay kit, such as BCA Protein Quantification Kit (ab102536).

What is the sample volume to be used with this kit for plasma samples from rat?

This depends on the amount of active CK enzyme in the sample. The sample volume per well would need to be optimized to make sure that the values obtained are within the linear range of the standard curve.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

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