

## ab211044 Lipid Extraction Kit (Chloroform-Free)

For chloroform-free lipid extraction from plasma, serum, cultured cells and tissues. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab211044](http://www.abcam.com/ab211044)  
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### Materials Supplied and Storage

Store kit at RT in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Lipid Extraction Buffer	25 mL	RT	RT
Lysis Buffer II	5 mL	RT	RT

### Materials Required, Not Supplied:

- Vacuum concentrator
- Dounce homogenizer
- Vortex

### 1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening

#### 1.1 Lipid Extraction Buffer

Provided ready to use. Store at RT and protect from light.

#### 1.2 Lysis Buffer II

Provided ready to use. Store at RT and protect from light.

### 2. Sample Preparation

#### 2.1 Plasma

- 2.1.1 Collect blood in a tube containing an anticoagulant such as citrate, EDTA, or heparin, and mix by inversion.
- 2.1.2 Centrifuge the blood at 1000 x g at 4°C for 10 minutes.
- 2.1.3 Collect plasma supernatant without disturbing the white buffy layer.
- 2.1.4 Samples can be extracted immediately or may be stored in aliquots at -80°C.

#### 2.2 Serum

- 2.2.1 Collect blood in a tube with no anticoagulant.
- 2.2.2 Allow the blood to clot at RT for 30 minutes.
- 2.2.3 Centrifuge at 2500 x g for 20 minutes.
- 2.2.4 Collect the supernatant without disturbing the white buffy layer.
- 2.2.5 Samples can be extracted immediately or may be stored in aliquots at -80°C.

#### 2.3 Cultured Cells:

- 2.3.1 Pellet 1-5 x 10<sup>5</sup> cells by centrifugation at 1000 x g for 5 minutes.
- 2.3.2 Wash cell pellet once with PBS.
- 2.3.3 Resuspend pellet in 25-50 µL PBS.
- 2.3.4 Perform the extraction as described below.

#### 2.4 Tissues

- 2.4.1 Carefully mince the tissue into small fragments with a scalpel/razor blade and weigh in a 50 mL conical tube.
- 2.4.2 Use ~10-50 mg of tissue (wet weight) per sample for extraction.
- 2.4.3 Perform the extraction as described below.

### 4. Assay Procedure

Equilibrate all materials and prepared reagents to correct temperature prior to use.

- Prepare samples as directed in the previous sections.
- The protocol below is for a 25 µL sample size. Number of preps per kit will decrease with increasing sample volumes.

**4.1** Add 25 µL of sample (or 10-50 mg tissue) to a clean 1.5 mL micro centrifuge tube.

**4.2** Add 500 µL of the Lipid Extraction Buffer to the sample.

**4.3** Immediately vortex for 1-2 minutes using a tube shaker or vortex.

**NOTE:** For tissues, homogenize the tissue pieces in a Dounce homogenizer on ice. Spin the homogenate at 10,000 x g for 5 minutes at 4°C and collect the supernatant.

**4.4** Agitate the mixture for 15-20 minutes on an orbital shaker at RT.

**4.5** Centrifuge the tube at 10,000 x g for 5 minutes and carefully collect the supernatant, which contains the lipids, and transfer to a clean tube.

**4.6** Record the volume.

**4.7** Leave the tube containing the supernatant open and dry it in a vacuum concentrator or in a dry 37°C incubator overnight

**NOTE:** A thin film can be seen after complete drying. The lipid sample can be processed further. If the lipids will be analysed by lipidomics, HPLC or MS, we recommend resuspending the lipid extract in an organic solvent such as butanol or cyclohexane. If the downstream application is analysis of lipids using assay kits (e.g., to quantify cholesterol, triglyceride etc.), we recommend resuspending the lipid extract in 50 µL of Lysis Buffer II. Sonicate the lipid extract for 15-20 minutes at 37°C.

### Technical Support

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