J.T.Baker® BAKERBOND®
SPE Silica-Based 96-Well Plates

SPE is an extraction process whereby the analytes of interest are removed from the sample matrix and trapped onto the sorbent. The analytes can then be removed by the application of a suitable eluting solvent.

THE MAIN STAGES OF SPE:

1. **CONDITION**
   To activate the sorbent a highly eluotropic solvent is applied to the sorbent bed, this ensures that the sorbent is fully wetted, and that the analyte will have access to the pore structure. Excess conditioning solvent is then removed by flushing the sorbent with a low eluting strength solvent, typically it will be the same solvent as is used to load the sample.

2. **LOAD**
   The sample is loaded onto the sorbent. The sample is loaded in a state that will optimise the retention of the sample onto the sorbent, which may require altering the pH of the loading solvent. It is important at this stage that the solubility of the sample in the loading solvent is considered carefully. At this stage some of the matrix components will be flushed through the cartridge as they will not retain on the sorbent. The nature of matrix components that are flushed from the cartridge will depend on the chemical nature of the matrix as well as the chemistry of the sorbent.

3. **WASH**
   A wash solvent is applied which is not strong enough to elute the compound(s) of interest from the sorbent, however it will remove some of the matrix components. This process can be optimised using a process called elution profile where differing eluotropic strength solvents are applied to determine the strength of the solvent required to elute the compound of interest. At this stage the impact of pH can be very important and may result in the use of two wash solvents at different pHs.

4. **ELUTE**
   The analyte of interest is eluted from the sorbent using a suitable solvent. Considerations should be made for the resulting eluent and making sure that there is compatibility with either a blow down step or the chromatographic procedure that will be applied directly after, as well as ensuring that the recovery is kept close to or ideally at 100%.

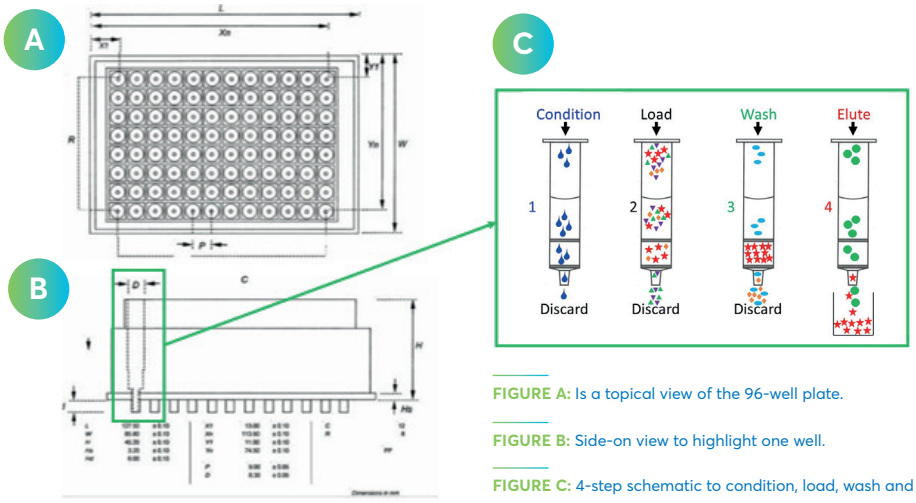


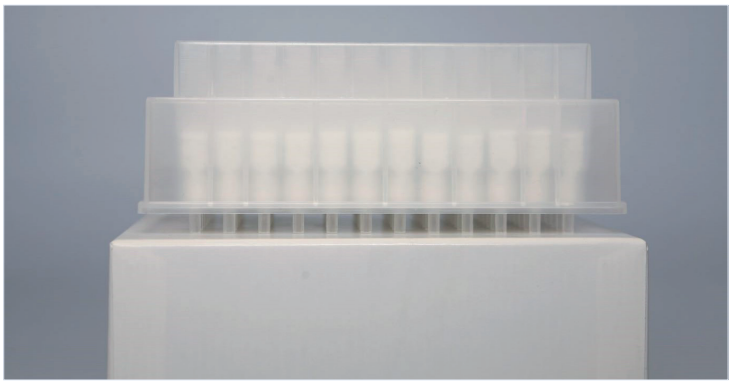
FIGURE A: Is a topical view of the 96-well plate.

FIGURE B: Side-on view to highlight one well.

FIGURE C: 4-step schematic to condition, load, wash and elute your sample of interest.

CARE AND USE

- J.T.Baker® BAKERBOND® spe products are designed to be single use
- Low and high pH can be used for silica-based single use SPE products if exposure is kept relatively short and low volumes are used
- Temperature recommendations: ambient
- Bonded stationary phases are stable up to 70 °C, however the technique typically uses organic solvents which have boiling points close to this value and hence this has to be the major consideration
- Pressure recommendations: <10 bars
- Solvent recommendations: compatible with typical HPLC grade solvents, please check solvent miscibility charts
- Solvent volume recommendations: limited by the physical dimensions allowable for each SPE well plate/cartridge volume. Please refer to validated methods/applications
- Sample load recommendations: a general rule of <5% sample to sorbent mass (mg) is recommended



PROTOCOLS

There will be differences in solvents and protocol depending on the sample matrix and application the following tables list some examples. For more information please visit **vwr.com/spe** or contact our Chromatography technical experts at **chromsupport@avantorsciences.com** for method development assistance.

Reversed Phase (C8, C18, C18 PolarPlus®)

Volume	10mg	30mg	60mg
Sample pre-treatment	Dilute sample in an aqueous environment with the addition of internal standards, this may be pH modified to ensure the target analyte(s) bind to the sorbent (not as critical compared to ion-exchange materials).		
Condition*	Organic solvent is added to each well. Followed by a very weak solvent to ensure binding of target analytes(s).		
Load*	Ensure the final mass of sample <0.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <1.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <3 mg using the same solvent used in the pre-treatment step.
Wash*	Using a stronger solvent than used in the conditioning, which does not elute the analyte of interest, wash and discard weakly bound analytes in the matrix.		
Elute**	Using a stronger solvent solution relative to the wash step to un-bind the target analyte(s) and collect.		

Ion Exchange (Aromatic sulphonic acid, Diamino (NH/NH_2), narc™-1, narc™-2, Quaternary Amine, Carboxylic Acid)

Volume	10mg	30mg	60mg
Sample pre-treatment	Dilute sample in an aqueous environment with the addition of internal standards, pH modified to ensure the target analyte(s) bind to the sorbent.		
Condition*	Organic solvent is added to each well. Followed by a very weak solvent to ensure binding of target analytes(s).		
Load*	Ensure the final mass of sample <0.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <1.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <3 mg using the same solvent used in the pre-treatment step.
Wash*	Using a stronger solvent which does not elute the analyte of interest wash the sorbent and discard weakly bound analytes in the matrix.		
Elute**	Using a solution specifically to un-bind the target analyte(s) and collect. If the sample was prepared in basic conditions, use an acidified aqueous solution in this step. If the sample was prepared in acidified conditions, use a basic aqueous solution in this step.		

Normal Phase (SiOH)

Volume	10mg	30mg	60mg
Sample pre-treatment	Dilute sample in a non-polar organic environment with the addition of internal standards, this may be pH modified to ensure the target analyte(s) bind to the sorbent (not as critical compared to ion-exchange materials).		
Condition*	A polar solvent is added to each well. Followed by a non-polar solvent to ensure binding of target analytes(s).		
Load*	Ensure the final mass of sample <0.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <1.5 mg using the same solvent used in the pre-treatment step.	Ensure the final mass of sample <3 mg using the same solvent used in the pre-treatment step.
Wash*	Non-polar solvent is used to wash the sorbent and discard any weakly bound analytes in the matrix.		
Elute**	Use a polar solution to un-bind the target analyte(s) and collect. If required, this may be pH adjusted to assist the elution.		

*Solutions used in this step are discarded. Up to 1000 µL volumes are allowable. Positive pressure or a vacuum can be applied to the 96-well plate to elute the liquid from the bed.
**It is recommended to limit the volume for a single step to <1000 µL, a validated assay/application may require blowing down this collected eluent from the final SPE step and reconstitution in a specified solvent environment.

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